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The bean anthracnose

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THE BEAN ANTHRACNOSE

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THE BEAN ANTHRACNOSE.

By C. W. Edgerton.

While the acreage that is used for bean growing in Louisiana is not large, the crop is a very important one in certain sections of the state. Favored with a semi-tropical climate, Louisiana can grow wax or snap beans and have them on the northern markets in early May or even the last of April, about the same time, or even before our sister states of the north are ready to plant. This, as a rule, means very good returns for the trucker, if he is situated where the transportation facilities are good and if he is able to keep his crop free from spotting.

The sections most used for bean growing are those joining the Illinois Central Railroad in Tangipahoa Parish, the region in the vicinity of New Orleans, parts of Livingston, East Baton Rouge and Plaquemines parishes.

While all parts of the state doubtless produce enough for home consumption, most of the beans raised on a commercial basis, are grown in the above regions. Tangipahoa is well adapted for bean growing, having a favorable soil and transportation facilities of the best. Beans shipped from this region can be placed on the Chicago market in 36 hours. The loss in transit is generally small. During 1909 the loss due to beans spoiling in transit, of those shipped from Ponchatoula, was about two per cent; and this small loss could doubtless have been reduced with proper care in picking and crating.

It is difficult to estimate the total acreage of beans in the state. W. S. Keaghey estimates that there were 240 acres in the vicinity of Ponchatoula alone. Perhaps a thousand acres would be a fairly accurate estimate of the spring crop during 1909. And the fall crop would probably raise this estimate somewhat for the whole year.

The total yield for the Ponchatoula region was 20,000 bushels of green pods or an average of 83 bushels to the acre. The maximum yield was about 140 bushels. The average return, with the transportation charges subtracted, was eighty-five cents per two-thirds bushel crate or an average return per

acre of $105. This is a very good return, considering that this represents only the spring crop and that the same ground is capable of producing another crop during the season.

The season of 1909, however, should be classed as a favorable one for bean growing. The weather conditions during the last part of April and the first part of May were excellent. There was practically no rain and the beans developed fast, and were in many cases almost free from spotting. During this dry spell, the greater part of the bean crop was picked and shipped. The beans that came on earlier and also those that came on later, however, formed under rainy and humid conditions and the loss from spotting was considerable. Years in which the pods develop during periods of very humid and wet weather give returns to the trucker, which are, to say the least, very discouraging. The pods spot, and of course become entirely unsalable. As a consequence, many truckers do not risk a very large acreage. If it were possible to eliminate the loss from the diseases, bean growing would rapidly become a more popular crop for the truck raiser.

The diseases to which beans are subject in Louisiana are as follows: Anthracnose, Blight, Rhizoctonia Rot, Sclerotium Wilt Disease, Cercospora Pod Spot, and Phyllosticta Leaf Spot. Of these the first five may be serious while the other two are of no importance from an economic standpoint. By far, the most serious of the troubles are the anthracnose and blight. As most of the work done at the Louisiana Station has been on the anthracnose, this alone will be treated in this bulletin; and as a popular bulletin was published on this subject in June, 1909 (Louisiana Bulletin No. 116), it has been thought best to treat the disease at this time in a technical manner only.

Before beginning the discussion of the disease, I wish to express my thanks to various people who have aided me in the investigation; especially to Mr. W. S. Keaghey of Ponchatoula for information in regard to bean growing and diseases; to Professor G. L. Tiebout, Horticulturist of the Station, for aid in carrying out the field work on the Station grounds; and to Professor H. H. Whetzel and Mr. M. F. Barrus of Cornell University for sending me a quantity of diseased and healthy seed for use in the investigation.

THE CAUSE AND DISTRIBUTION OF THE DISEASE.

The bean anthracnose has been known to botanists and horticulturists for about thirty-five years. It was first noticed in Popplesdorf in 1875 by Lindemuth. Whether it existed to any extent before that is unknown, but it is somewhat questionable, as it probably would have been noticed by some of the early botanists or horticulturists. Soon after the first notice of the trouble, it began to be a very important factor in bean raising. At present it is cosmopolitan in nature, being present in practically every country in the world. At the present time, there are but few diseases which are more familiar to plant pathologists and horticulturists than this. As it causes a large financial loss every year, it has been the subject of considerable study at various times by different workers.

The disease is due to the fungus, *Colletotrichum lindemuthianum*. At first it was named *Gloeosporium lindemuthianum*, but since the presence of setae in the fruiting postules was demonstrated, the former name has been mostly used. The disease appears as spots on all parts of the bean plant above the ground, and sometimes even extends a short distance below the surface of the ground on the stems. As the appearance of the disease on the different parts of the host plant is fairly familiar to botanists, I will only give a short description of it.

THE SPOTS ON THE PODS.

The spots on the pods are the ones most frequently noticed by the bean grower. The spots show first as very small dark colored places on the pods but they increase in size very rapidly, sometimes becoming a centimeter in diameter. Often also, different spots will coalesce and form very large lesions, these sometimes covering the greater part of the pod. The spots (Plate 1) are as a rule round or oval, but sometimes vary into irregular shapes. Soon after the spot is visible on the pod, it becomes darker in color and the tissue dries up and shrinks and as a result is depressed. The fully developed spot is considerably sunken, nearly black in color in the center, and generally surrounded by a reddish zone. Soon after the spot becomes shrunken, the acervuli or fruiting pustules begin to develop, and the

surface takes on a pinkish color from the mucilaginous mass of spores formed. Also at this time, the spots are generally more or less slimy.

THE SPOTS ON LEAVES.

On the leaves, the disease appears almost wholly on the veins and petioles. The spots are generally seen on the under side of the leaf alone, due without doubt to the fact that the source of infection is generally from beneath. The affected veins become black in color and the larger ones somewhat sunken, similar in this respect to the spots on the pods. The fungus grows rapidly along the larger veins and also out on the smaller veinlets (Plate II). If the infection is severe, sometimes the leaf tissue dies between the infected veins, but normally in the field this seldom occurs. Often also, the disease attacks the small growing leaves causing them to become twisted and crinkled (Plate V, fig. I). This is due to the continued growth of the parenchymatous tissue of the leaf after the veins are dead.

While infection generally takes place in the field on the lower side of the leaf, the upper side is just as susceptible to the disease if the spores are present. Experiments were tried in the greenhouse to see if either side of the leaf was more easily attacked by the fungus. On some leaves, spores were sprayed on the under side with an atomizer, while on others, they were sprayed on the upper side. After the normal period of incubation, the spots appeared abundantly on all of the leaves, but only on the side on which they were inoculated. On the leaves sprayed on the under side, there developed typical anthracnose spots on the veins, taking some time for the disease to grow through the leaf so that it would show on the upper side. Leaves sprayed on the upper side, had the veins killed on the upper side alone, and the typical spots which normally appear on the lower side of the leaves were absent. The veins on the upper side did not take on the deep black appearance that is so often seen on the lower surface but were more brown in color; otherwise the spots were similar. From this we are forced to believe that the leaves are normally infected from the lower side, probably due to a considerable extent to the splashing up of the spores from the ground.

THE SPOTS ON THE STEMS.

On the stems, the spots sometimes develop profusely. On young seedlings the spots are generally below or at the point of attachment of the cotyledons due to the spores being washed down from the diseased cotyledons. On older plants, the spots are more scattered over the different parts of the stems. The spots appear quite suddenly on the stems and differ but little from those on the pods (Plate IV). However they are as a rule elongated in the direction of the main axis of the stem. Sometimes, also, there are dark streaks extending up and down from the spots. These streaks are quite distinct near the spots but gradually fade out in the healthy tissue of the host. The spots are black and slightly sunken, and, as on the pods, become covered with the pink, slimy exudate of spores. By coalescence, the spots may form large lesions three or four inches in length. As these large lesions grow older, they dry out and the tissue cracks (Plate VI). Often on young plants, the disease becomes so bad that the stem completely rots and we have a "damping off" effect (Plate IV). On older parts of the stem, when the tissue becomes hard, the spots rarely form; and when they do, they remain small.

THE SPOTS ON THE SEED.

On the seed, the disease appears also in yellowish, brownish, or blackish spots. These are located directly under the spots on the pods and are formed by the fungus growing down through the pod tissue and into the seed. Generally the spots are rusty brown or black, but if the disease has just reached to the seed, merely a yellow spot may be formed. The spots are various in size from a mere speck up to one including the whole side of the bean. As a rule, they are round, but may vary into irregular shapes. These are also, as a rule, surrounded by a reddish zone. The spots may or may not be sunken and may or may not contain acervuli and spores. This depends entirely upon how badly affected the seed may be.

THE SPOTS ON THE COTYLEDONS.

The spots on the cotyledons are the same as those on the seed. When the seed absorbs water in germination, the fungus

also begins to grow. While on the seed, we have the fungus in a dormant condition; in the spots on the cotyledons, we have the fungus in a growing condition in the same spots. The spots enlarge, become more shrunken, and develop spores in abundance. The spots are quite similar in appearance to those on the pods.

MICROSCOPIC STRUCTURE OF THE SPOTS ON THE PODS.

When the spots are first noticed on the pods, they are small, yellowish, or slightly brownish in color, and round or somewhat oval in shape. At first, these spots are not sunken, but become slightly so in twenty-four hours after being first visible. The spots are always more or less localized. Even in sections of young spots, it is generally an easy mattery to see the boundary between the diseased and healthy tissue.

The structure of the healthy bean pod is as follows: On the surface there is the epidermal layer of cells of the ordinary rectangular shape, thin walled except on the outside where there is a rather thick, and very much corrugated cuticle. Under the epidermal layer, there is a tissue made up of thin walled polygonal cells from ten to twenty cells in thickness. Below this there are a few scattering, poorly developed fibro-vascular bundles and under these a rather dense layer of very small cells placed very close together. This last layer is only three to five cells in thickness. Under this again is a thick layer of large polygonal thin walled cells.

A section now of a young anthracnose spot shows the cells unchanged in shape, but the upper layer of large polygonal cells contains an abundance of mycelial threads of the fungus and very little of the original protoplasmic contents of the cells. The mycelium stains deeply and appears very prominent when stained with Iron Alum Haematoxylin. A small region on the boundary between healthy and diseased tissue is made up of cells which have also lost most of their protoplasmic contents but which as yet contain very little fungus mycelium.

When the spot is about twenty-four to thirty-six hours old, the outer cells begin to collapse and as a result the spot becomes

shrunken. Figure 1, in Plate XII, shows cells that are just beginning to shrink. The cells in the lower part of the figure are still polygonal and normal in shape but those near the surface are becoming flattened. During the development of the spot, the mycelium continues to spread in all directions. However when it reaches the thin layer of small cells its inward development seems to be slightly checked. In some cases, it does not seem to be able to pass this layer of cells, but in most cases, if the conditions for development are good, the mycelium grows through these cells and into the large ones beneath.

The cells in the tissue just underneath the epidermis dry out very rapidly, and in spots two to five days old, the cell walls in many cases are lying side by side (Plate VII, fig. 2). At this stage the boundary line between the living and dead cells is very distinct, as is shown in the figure.

The action of the fungus on the deeper layer of large polygonal cells is somewhat different from that on those just underneath the epidermis. The outer cells, as has been said, dry and shrink, but the inner ones are more or less protected from this shrinking. But the fungus seems to disintegrate these almost entirely. A section at this time will generally show a large empty cavity (Plate VII, fig. 2) between the outer layers and the healthy tissue near the bottom of the spot. The accompanying photomicrograph is very typical of old anthracnose spots on the pods, it showing the sharp boundary between healthy and diseased tissue; the dead, deeply staining cells, packed full with fungus mycelium and apparently in contact with healthy ones; the shrunken cells of the outer layer of the pod; and the disintegrated cells of the inner layer.

The development of the acervuli in these spots will be considered later under the development of the fungus.

MISCROSCOPIC STRUCTURE OF THE SPOTS ON THE SEEDS.

The microscopic study of the diseased seed presented some interesting information. On account of the thicknness of the cell walls, the density of the cell contents, etc., the cutting and staining of sections is rather unsatisfactory. But some fairly

good sections were obtained. These showed the spots to be localized similar to those on the pods. As the diseased portion takes the stain very deeply, there was a sharp contrast between this portion and the healthy tissue. The diseased cells are more or less shrunken and poorly developed.

Acervuli are often present on the surface of the spots and also between the cotyledons. Also some of the sections of diseased seed showed very interesting fruiting pustules buried in the tissue of the bean. These (Plate XIII, Figs. 2 and 4) were closed pycnidial like bodies buried some distance beneath the surface of the bean. It appeared as if the fungus had caused a spreading apart of some of the layers of cells and then formed a conidiophore layer around the margin of this cavity. These pycnidia were packed full of spores which seemed to be in a perfectly healthy state. Two of these pycnidial like cavities are shown in the photomicrograph in Plate XII, Fig. 2. These are the two more or less clear places lying in a line about an inch from the upper edge of the figure. Figure 4 shows one of these under greater magnification. While this is not very clear, it shows the parallel conidiophores on all sides of the cavity and the abundance of spores in the center. These pycnidial-like fruiting bodies vary in size, as I have seen them, from 80—130x80—520 microns.

NORMAL LIFE HISTORY OF THE FUNGUS.

The bean anthracnose, while it follows in a general way the life history of other anthracnoses, differs in some of the details. The different steps in the life history of the fungus are more or less familiar to plant pathologists, and it will not be necessary to give a very extended discussion at this time, and so I will only briefly trace the normal development of the fungus through its different stages. The spores, which may be used for a starting point, are developed in all of the spots previously described. These are imbedded in a gelatinous matrix and are only disseminated by being washed off by rain or dew, or by being rubbed off. As long as the spores are imbedded in this mucilaginous substance they will not germinate, but when they are washed out of this and are placed in a moist place they

quickly germinate. If the spore comes to rest on some part of the bean plant, as for instance a pod, and if the pod is moist, as it generally is at times when the spore would be placed there, it quickly sends out a germ tube at a point near the end of the spore. This tube only grows for a short distance when there is formed at its end, a dark brown cell known as a secondary spore or appressorium. This latter spore soon germinates and forms a small tube which bores its way through the epidermal cells of the host plant. If a surface section of a pod is examined some 12 to 24 hours after it is sprayed with a suspension of spores in water, a considerable number of the appressoria will be seen. I have tried to get cross sections, both by free hand and with the microtome, that would show the entrance of the germ tube, but from the lack of a good differential stain, I have not been able to get one to show satisfactory. Some of them showed indistinctly, but not clear enough to be photographed or drawn. After the fungus has entered the host tissue, it bores its way from cell to cell, passing directly through the cell walls. The cells become filled with mycelium and after the normal period of incubation they die and collapse. The fungus continues to grow, spreading rather rapidly at first, but later for some reason, the growth is checked and the fungus remains localized. The mycelium does not extend out further than the host tissue is discolored.

After the host tissue is killed, the fruiting pustules of the fungus develop. In the development of the acervulus, there is at first a collection of a few large mycelial threads in the epidermal and sub-epidermal layers of cells (Plate XII, fig. 1). These few scattering threads quickly give rise to a number of conidiophores lying side by side and nearly perpendicular to the surface of the pod (Fig. 2). Generally in descriptions of the development of the acervulus, authors state that the formation of the conidiophores is preceded by the formation of a stroma. Beach (2) says: "The mycelium * * * just beneath the epidermis * * * forms a dense dark colored stroma which precedes the production of spores." A glance at Fig. 2 will show that no stroma precedes the development of the conidiophores. These are only preceded by a few scattered threads as

shown in figure 1. These conidiophores, while few at first rapidly increase in number (Fig. 3, 4, and 5). These develop in the two or three layers of cells underneath the cuticle. They also increase in length, raising the cuticle of the host plant and finally rupturing it, though the rupturing seldom occurs before a few spores begin to form. Often there is a later development of a stroma of pseudo-parenchymatous cells (Fig. 6).

Spores very soon form after the conidiophores develop. A constriction is formed a short distance back from the apex of the conidiophore and the apical portion, which is now the spore, is cut off. Spore after spore is cut off from each conidiophore and in a short time there is a mass of spores at the surface of the acervulus. Although the cuticle is ruptured soon after the spores begin to develop, it still holds the spores more or less confined, and they are only forced out of the small opening formed, (Plate XIII, fig. 1). Later, however, the cuticle is thrown entirely aside, and the mass of spores takes on the appearance as shown in figure 4. The spores are held together at the surface of the acervulus by the mucilaginous substance secreted by the fungus.

Spores are formed in great numbers and very rapidly. I estimated the number of spores on the pod farthest to the right in Plate I. This pod is quite severely effected, but no worse than many pods in the field. On bringing the pod in, I washed off all the spores in a definite amount of water. By diluting this suspension of spores and making poured plates from a very small per cent of it, I estimated the number of spores on the pod as between 110 and 115 million. The pod was then placed in a moist chamber for 24 hours and spores allowed to develop again. At the end of this period, there were again between 45 and 50 million spores on the pod. It is safe to say that some pods may develop from one-half to one billion spores during their growth.

After the spores begin to form, quite frequently there develop a few scattering setae in the acervulus. However these are not always present, and it was some years after the bean anthracnose was discovered before these were seen (28). These are as a rule slightly more abundant than in the related form on apple, but less abundant than in the form on cotton.

If the spot on the pod is directly over a developing seed within, and if the infection has been early and conditions are suitable for a good development of the fungus, often the mycelium penetrates entirely through the pod tissue and through the seed coats into the seed itself. The tissue of the seed is killed in the same manner as that of the pod. The size of the spot formed on the seed depends upon how near mature the seed was when first affected, and upon temperature conditions. If the seed becomes infected when it is young, the spot may cover half of the seed and may grow to the center of the seed or even beyond. If the seed becomes infected later, the spot remains smaller and shallower. A common condition is where the discoloration is in the seed coats alone, the cotyledons below, when exposed, appearing normal. Large spots on the seeds generally contain spores in abundance on the surface. Spores may also develop abundantly between the cotyledons and also in buried pycnidial structures in the tissue itself as described above.

In cases where the disease does not pass entirely through the pod tissue, there is often a lack of development of the tissue of the seed directly under the spot. An examination of a large number of seed from directly under anthracnose spots, seed that showed no infection, not even any discoloration, showed a large per cent to have depressions at the point nearest to the spot on the pod. It would appear from this that the developing seed draws nourishment from the pod from all sides, and if some of this pod tissue is used up by the fungus, that portion of the seed nearest to the diseased portion of the pod naturally fails to develop. These depressions in the seed were sometimes a millimeter in depth though generally shallower.

The spots on the leaves and stems develop in a smilar manner to those on the pods.

Of course any portion of the plant may be infected from any other diseased portion, but the usual course of the infection is as follows: The disease, being present in the seed, continues to develop on the cotyledons. By the time the cotyledons are above the ground, the spores are generally abundant in the spots. These are washed off with the rain and dew and pass down on the stems and ground. Finding lodgment on the stems,

they quickly cause the spots there. Spots on the stems above the cotyledons are rare on young plants. The leaves above, then become infected by being blown against the diseased cotyledons or stems, or the ground, or by rain splashing up the spores from the ground on to them. Spots form on the leaves and the spores are washed down from these on to the higher parts of the stems and the pods.

If the different plants in the field are in contact, the spread of the disease may be rapid, but if they are separated, the spread of the trouble is very materially checked. While the disease may be spread by insects in some cases, there is no evidence to prove it. I have seen large patches of beans less than 100 yards apart, where one patch would be badly affected and the other without any anthracnose whatever. The disease is without doubt spread most frequently through different parts of the field by the carelessness of the growers in walking through or working the fields while the plants are wet with rain or dew. The disease may also be spread by a severe storm which whips the plants a great deal.

The ascogenous or perfect stage of the fungus has never been observed, as far as I know, on plants in the field. Shear and Wood (29), however, have reported it in pure cultures on artificial media. I have tried many times to get a strain that would produce the perfect stage but as yet have been unsuccessful. The perfect stage of the bean anthracnose needs to be studied very carefully. The other characters of the fungus are so different from the anthracnoses from other hosts, that it would not be surprising if the characters of the perfect stage would also be slightly different.

PERIOD OF INCUBATION.

Frank (10) claims to have secured brown spots and mycelium 24 hourse after infection with the fungus. Halsted (17) states that he procured infection after 36 hours. But from very carefully conducted experiments, I am unable to agree with either statement. The lowest period of incubation which I have been able to obtain was four and one-half days. Longer periods than this are generally the rule, especially if the weather and

temperature conditions are not quite favorable. In determining the period of incubation, I have used plants in both field and greenhouse. All the inoculations were made by spraying on the plants a suspension of spores in water by means of an atomizer. No experiments were tried with needle punctures as the disease does not seem to gain entrance through wounds. Various parts of the plants were inoculated, but the period of incubation seemed to be the same on pods, stems, and leaves. Two different cultures were used in these experiments, both obtained from germinating seed, one isolated in November, 1908, and the other about February 1, 1909. These two cultures were identical and when used at the same time gave the same amount of infection and the same period of incubation. The following tables show the results of the experiments tried to determine the period of incubation. The dates given are the ones in which the first spots were able to be seen. The plants were examined about twice or three times a day after they were inoculated. In all cases, unless otherwise stated, on the first or second day following the date given in the tables, the pods and all young parts of the plants were covered with spots. The pods in Plate I were taken from some of the infection experiments.

Table 1 shows the results of inoculations in the greenhouse. I have included the dates, the number of plants used in each case, the parts inoculated, and the treatment of the plants. By treatment, I mean the length of time that the plants were covered with bell jars. In all cases, except one, the inside of the bell jar was covered with wet paper. Two sets of plants were inoculated without being covered with jars. Of all of the inoculations conducted, these were the only ones in which I failed to get an abundant infection.

TABLE NO. 1.

GREENHOUSE INFECTIONS.

No. of Plants.	Date of Inoculation.	Period Covered.	Parts Inoculated.	Date First Spots.	Period of Incubation.	REMARKS.
4	Mar. 22, P. M.	40 hrs.	Levs. and Stems.	Mar. 27, P. M.	5 days.	
6	Mar. 26, P. M.	40 hrs.	Lvs., Sts. and Pods.	Mar. 31, P. M.	5 days.	
2	Mar. 24.	0	Lvs., Sts.	Apr. 3.	9 days.	Slight Inf.
2	Mar. 24.	16 hrs.	Lvs., Sts.	Apr. 1.	7 days.	
2	Mar. 24.	24 hrs.	Lvs., Sts.	Mar. 29.	5 days	
2	Mar. 24.	40 hrs.	Lvs., Sts.	Mar. 29.	5 days.	
2	Mar. 24.	60 hrs.	Lvs. Sts.	Mar. 29.	5 days.	
2	Apr. 5. P. M.	40 hrs.*	Lvs. Sts.	Apr. 10, P. M.	5 days.	
4	Apr. 5, P. M.	40 hrs.	Lvs. Sts.	Apr. 10, A. M.	4½ days.	
2	Apr. 5, P. M.	0	Lvs. Sts.	....	.....	No. Inf.

*The bell jar covering these plants had no lining of wet paper.

The lowest period of incubation obtained in these experiments was four and one-half days, and the conditions in most of them were the best possible for the development of the fungus. Where the plants were not protected from drying, longer periods of incubation were obtained. The greenhouse was very dry and those plants that were not covered at all gave very slight infection, one set not becoming infected at all and the other not until the end of nine days. Sixteen hours was also not quite sufficient for the fungus to get thoroughly established before the plants became dry. But plants covered for 24 hours were as badly infected as those covered for 60 hours. To get the best infection, it would seem, that we should have about 24 hours of rainy weather. In Louisiana, where we have heavy dews which stay on the plants until nearly eleven o'clock, we have conditions approaching the optimum for the fungus.

The field inoculations were mostly carried on during a rainy spell. I wanted to try some during a dry spell to test the effect of the dews alone, but I did not have an isolated patch in good shape for inoculation during the dry spell we had during the last of April. In each experiment ten hills of beans

were used, there being on an average of three plants to the hill. Each plant contained from fifteen to twenty-five pods. Altogether there were nearly 400 plants used in the field inoculations. In all of the infection experiments, both in the greenhouse and in the field, check plants were used, but in no case did spots develop on these. In Table No. 2, I have given the date of inoculation, date of first spots, weather conditions, and age of the culture used for inoculation.

TABLE NO. 2.

FIELD INFECTIONS.

Date of Inoculation.	Age of Culture.	Weather 24 hours previous.	Weather 24 hours following.	Date of first spots.	Period of Incubation.
May 15, 12 Noon	15 days.	Warm, dry.	Warm, dry.	May 21, Morning.	5½ days.
May 16, 9:30 A. M.	16 days.	Warm, dry.	Shower, cloudy.	May 21, Morning.	5 days.
May 17, 9 A. M.	17 days.	Shower, cloudy.	Warm, showers	May 22, 6 P. M.	5½ days.
May 17, 5 P. M.	17 days.	Showers.	Rain, warm.	May 23, 10 A. M.	5½ days.
May 18, 9 A. M.	18 days.	Rain.	Warm, shower.	May 24, 9 A. M.	6 days.
May 18, 5:30 P. M.	4 days.	Rain.	Rain.	May 23, P. M.	5 days.
May 19 9 A. M.	5 days.	Rain.	Warm, sunshiny.	May 24. 6 P. M.	5½ days.
May 19, 6 P. M.	5 days.	Warm, sunshiny	Heavy dew. cool.	May 25, 9 A. M.	5½ days.
May 20. 9 A. M.	4 days.	Cool, heavy dew.	Warm, shower.	May 25, 6 P. M.	5½ days.
May 20, 6 P. M.	4 days.	Warm.	Shower.	May 25, 6 P. M.	5 days.
Map 21, 12 Noon	4 days.	Shower	Sunshiny.	May 26, 8:50 A. M.	4 5-6 days
May 21, 6 P. M.	21 days.	Warm.	No rain.	May 26, 6 P. M.	5 days.

The period of incubation in the field tests runs from slightly under five days to six days. The weather conditions were not variable enough to give any variation in the period of incubation, or else the heavy dews were sufficient to cause abundant infection in spite of any lack of rain. The weather during the inoculation experiments was quite warm during the days, but with cool nights.

It is hard to understand how Frank and Halsted obtained the results they did, unless they used plants which were already previously infected. It would be impossible to conduct such experiments in a field where there was any disease present, as the results would not be reliable. The above described experiments were conducted upon plants grown from clean seed, and at no time did any plants become infected other than those inoculated.

GROWTH IN CULTURE MEDIA.

Cultures have been made of this fungus at various times from different localities and from different parts of the host plant. The various points which can be studied by the use of cultural technique have been considered; as the germination of spores under various conditions, cultural characters, spore and appressoria formation, variability of cultures, etc. Various culture media have been used during the course of the study including potato, bean, and rice agars; bean pods; alfalfa and Egyptian clover stems; cornmeal; Prazmouski's nutrient solution, etc. The culture work has been conducted in the usual manner.

GERMINATION OF SPORES.

The characters of spore germination vary with the culture media used, the age of the spores, and the number of spores in the liquid in which they are germinating. A spore in pure water germinates after a few hours by sending out a small germ tube which only grows for a short distance when there is formed at its end an appressorium, the contents of the spore and germ tube passing gradually into the appressorium. Sometimes a second germ tube may be formed, but this is not the rule. If the water used contains a small amount of food material, as ordinary tap water generally does, the germ tube is longer. In these cases, where the amount of food material is relatively small, the germ tube is generally narrow. Often, also, under these conditions, when there are a number of spores in the medium, the germ tubes will anastamose (Fig. 12, Plate XIV). The per cent of germination in water is often low, and rarely do all of the spores germinate at once. A few will germinate in a few hours while some will not germinate for a day or more. Where the

surrounding medium is rich in organic food material, the germination is generally simultaneous.

In ordinary nutrient media, if the spores are scattering, they germinate by sending out germ tubes slightly smaller than the spores themselves. These tubes are sent out, as a rule, from near the end of the spores, and at a slight angle to their main axis. The first germ tube is often followed by one or two others from different parts of the spore. These absorb the food from the culture medium and soon there is developed a much branched and septate mycelium. The walls of the mycelial threads at this time are thin and hyaline, and the protoplasmic contents somewhat granular. The identity of the spore soon becomes lost in the network of mycelium that is developed. The spores swell but very slightly, if at all, in germination under these conditions.

However, if the spores are very abundant and close together in the culture medium, a different sort of germination results (Plate XIV, fig. 5 and 6). There is at first a swelling of the spore, with generally the formation of a septum through the center. Further swelling of the spore after the formation of the septum, causes the spore to become constricted at that point (Fig. 6). The spore sometimes swells to a size two to three times that of the normal one. Often from these large swollen spores, germ tubes of various sizes are sent out. These, however, seldom grow to any great length. This sort of germination is very similar, if not identical, with that described by Atkinson (1). I made a number of plates with varying numbers of spores in them, the number of spores being estimated in each case. The spores were then allowed to germinate with the following results:

150 spores to cubic mm. of medium. Spores very much swollen and septate with but few germ tubes.

75 spores to cubic mm. of medium. Spores much swollen and septate, with about 25 to 50 per cent with germ tubes.

30 spores to cubic mm. of medium. Spores more or less swollen and septate, with about 50 to 90 per cent with germ tubes.

16 spores to cubic mm. of medium. Spores but slightly swollen with nearly 100 per cent with germ tubes.

12 spores to cubic mm. of medium. Very similar to the preceding.

6 spores to cubic mm. of medium. Practically no swelling with germination normal and practically 100 per cent.

From this we see, that when the spores are crowded, that is, when there are more than 12 or 15 spores to the cubic millimeter of medium, the spores become swollen, germinate slowly and but poorly. When the number is less than this, they germinate with but little, if any, swelling. Whether the spores give off an enzym in germination which prohibits growth is questionable, but it seems reasonable.

The spores that swell and germinate in this way, often act in a peculiar manner later. The germ tubes, if they do develop, only grow for short distances. Some of them stop growth at this point with no further change. Often, however, spores are again formed on these short germ tubes (Plate XIV, fig. 7). Often, also, spores may develop directly from the swollen spore without the previous development of a germ tube (fig. 7). The spores that develop in these ways are perfectly normal, appearing identical with those that develop in acervuli on bean pods.

Another variation appeared in the germination of old dried spores. Some experiments, which will be discussed later, were tried to see how long dried spores were viable. These spores, just before they lost their viability, germinated slowly in nutrient media. Although they were not crowded, they swelled somewhat and then sent out very large germ tubes, as large as the swollen spores themselves (fig. 8). These grew slowly at first, but later took on the ordinary method of growth.

DETERIORATION OF SPORES.

A spore when first formed, is composed of an apparently homogenous, granular protoplasm, with the exception of the small, round, clear nucleus near the center. In cultures, these spores form in large pink masses, retaining this color for from one to three weeks or even more, and then gradually fading out to a cream color. If examined microscopically during this change of color, a change in the contents of the spores themselves will be observed. The protoplasmic contents, which at

first completely fill the spore, gradually shrink and vacuoles of various sizes are formed (Plate XIV, fig. 2). This shrinking of the protoplasmic contents continues, the vacuoles growing larger until there is little left beyond a rim of protoplasm lining the wall of the spore. As deterioration continues, the protoplasm seems to go to pieces, and becomes collected in balls and masses throughout the interior of the spore (fig. 3). In some spores, the protoplasmic contents seem to entirely disappear. Germination tests were made of the spores in these different conditions. Spores that are merely vacuolate are still capable of germination. They absorb water from the nutrient medium causing the vacoles to entirely disappear, and as a result becoming perfectly normal in appearance. They then germinate in the usual manner. However, the spores in which the protoplasm has broken away from the edge and collected in masses, are not capable of assuming their ordinary appearance or of germination.

FORMATION OF APPRESSORA.

The large dark cells formed by various forms of anthracnoses and called appressoria or secondary spores (Plate XIV, fig. 10 and 11) are developed by the bean anthracnose at various times. As has been mentioned, they form on the bean pods previous to infection and also in germination in pure water. They also form occassionally in pure cultures. Here they do not necesarily develop in contact with the glass of the petri dish or tube. In crowded cultures, they often form in the agar entirely distinct from the glass or any solid substance in the media. In appressoria which I have observed from the bean anthracnose, the clear spot near the center, the so-called "germ pore" has often been lacking.

CULTURAL CHARACTERS.

The cultural characters of the bean anthracnose are quite distinct from all other members of this group which I have studied. The only other one that I have found that has characters approaching them is one which I found on Ludwigia alternifolia at Baton Rouge, and this could hardly be said to be similar. The two most distinguishing characters of the bean anthracnose are the more or less localized growth and the deep

black color which appears in cultures a few days old. If a germinating spore is placed in the center of a petri dish containing sterile agar, the resulting growth seldom extends out more than a centimeter from the initial point. The growth is at first white but soon takes on a jet black color. The growth is generally nearly strict, though occassionally there may be a little aerial growth. The spores form on this black substratum as a pink slimy covering or in pink pustules. In tubes containing sterile bean pods or stems of various plants as alfalfa or cowpeas, the mycelium may finally cover the whole exposed surface though the growth is generally comparatively slow. On all culture media, on which I have grown this form, the deep black color appeared.

Of all the cultures I have made of the bean anthracnose from various localities and from various parts of the bean plant, I have seen no differences in the culture characters. Furthermore, cultures which have been kept for a year or more have shown, as far as I could see, no variation. Many other anthracnoses are extremely variable as has been shown in a previous paper (6). Perhaps the anthracnoses which have shown the greatest variability are those which seem to have lost the power to produce the ascogenous stage, while the most constant ones are those which readily produce this stage. Reasoning from this, I have often thought that the bean anthracnose probably has the ascogenous stage developing at times.

MYCELIAL CHARACTERS IN PURE CULTURE.

In a young culture of the bean anthracnose, the mycelial threads are hyaline, small, and more or less of the same size. But as the culture grows older, these small threads are displaced by much larger filaments. These filaments are somewhat irregular and more or less broken up. The individual cells are more or less rounded and coarsely granular (Plate XIV fig. 14). These remind one very much of some of Viala and Pacotett's (35) figures and descriptions in their articles in which they attempted to show that the anthracnoses had yeast forms in their life cycle. These large filaments gradually darken in color and become thick walled (Plate XIV, fig. 13). These black threads com-

pose the stroma or crust which is seen on all old cultures. While this may not be a true stroma, it has many of the characteristics of one. This black growth is often pseudo-parenchymatous in character.

PERIOD OF VIABILITY OF SPORES.

The spores of the anthracnoses are supposed to lose their viability in a short time, especially after they have become freed from the mucilaginous substance in which they are imbedded. Experiments were undertaken to test this with the bean anthracnose. Two lines of investigations were undertaken: 1st, to see how long spores that developed on the bean seed would remain viable; and, 2nd, to see the effect of drying on the viability under various conditions.

The experiments from seeds and pods were as follows:

In March, 1908, I obtained diseased seed in the market, and placed some of the spores in drops of sterile water and left them to germinate. No spores germinated.

In March, 1908, I placed some spores from pods that were picked in May, 1907, in drops of sterile water and left to germinate. No spores germinated. These two tests were hardly fair ones, as fresh spores do not germinate very well in pure water. Furthermore, only a relatively small number of spores were under observation.

In February, 1909, I again obtained diseased seed on the market, without doubt seed of the 1908 crop. Cultures in potato agar were made from the spores on the spots on three beans. From one bean, no spores were seen to germinate, but from the other two, a few scattering spores germinated and grew into the typical colonies. Cultures from these gave good infection when sprayed again on young bean plants.

Dilution cultures were made at the same time from spores that had developed between the cotyledons in the seed. These gave a very high per cent of germination, nearly as high as fresh spores. As planting time for beans in Louisiana is in March, it is evident that some of the spores on the diseased seed are still capable of germination at that time, and it is possible that some of the infection on the young plants come from these.

These spores are rubbed off the diseased seed to the healthy ones, and when the beans are planted, germinate and infect the young plants. It is barely possible that this is one of the reasons why the picking out of diseased seed has been a failure from the standpoint of the control of the disease. Perhaps the picking out of the diseased seed, combined with some seed treatment to kill any spores on the surface, would give better results. It has been taken for granted that the seedling infection comes entirely from diseased seed, but from the germination tests just given, a second method, the infection from spores must be taken into consideration.

Tests were made in the spring of 1909 to see how long spores would remain viable when they were allowed to dry down in their own mucilaginous covering, and also how long when they were freed from this. Hasselbring (20) in working on the bitter rot of apples, found that spores washed free from the mucilaginous matrix and allowed to dry, would not germinate in water a day or so later; while those that were left imbedded in this substance retained their power to germinate for some time. As bean anthracnose spores do not germinate extra well in water, for the following tests nutrient media was used. Spores in all cases were obtained from pure cultures.

In order to dry the spores thoroughly and also to keep them away from any contamination, they were placed on sterile cover slips and placed in petri dishes that had been previously sterilized. Spores were placed on these cover slips in three different methods as follows:

Lot No. 1. A mass of spores was placed directly on the cover slip, and these were allowed to dry down in their own mucilaginous matrix.

Lot No. 2. Spores were placed in drops of sterile water on the cover slip and the water allowed to evaporate. This at least partially freed the spores from the mucilaginous covering.

Lot No. 3. Spores were placed in drops of sterile potato bouillion on the cover slips and allowed to dry.

A number of cover slip preparations were made in each lot. These were left in the petri dishes in the laboratory for different periods. To test for germination, a cover slip was dropped in

a tube of melted agar, the spores rubbed off in the medium, and a poured plate made. In the following table the results of the germination after different periods of drying is given:

TABLE NO. 3.

EFFECTS OF DRYING ON GERMINATION OF SPORES.

Lot. No.	Time Dried	Number of Plates	RESULTS
1....	2 days	1	Some germination. Better than two following.
2....	2 days	1	Some germination. Better than next following.
3....	2 days	1	Some germination.
1....	6 days	1	A few spores germinated.
2....	6 days	1	A few spores germinated.
3....	6 days	1	A few spores germinated.
1....	17 days	3	2 plates, no colonies; 1 plate, about 100 colonies.
2....	17 days	3	No germination.
3....	17 days	3	2 plates, no colonies; 1 plate, 1 colony.
1....	30 days	5	No germination.
2....	30 days	5	No germination.
3....	30 days	4	No germination.

While the per cent of germination decreased in all quite rapidly, very little difference, if any could be observed in the different lots. In seventeen days only a very small per cent of any of the spores were viable and after thirty days there were none.

In order to free the spores entirely from the surrounding mucilaginous covering, a second experiment was conducted. Spores were placed on sterile cover slips in sterile petri dishes as in the previous experiment but the preliminary treatment of the spores was somewhat different. They were treated as follows:

Lot No. 1. Spores were placed directly on the cover slips and allowed to dry down in their mucilaginous covering.

Lot No. 2. The spores were first placed in sterile water in a previously plugged and sterilized sedimentation tube. They were then centrifuged for about three minutes. By this method, the spores were freed from the mucilaginous covering and were also thrown down in mass in the bottom of the tube. The water was then poured off and the spores from the bottom were placed on the cover slips and allowed to dry.

Lot No. 3. The spores were centrifuged as in Lot No. 2, and were then placed in drops of potato bouillon on the cover slips and allowed to dry.

The spores were dried on February 15, 1909. Cultures were made the same day from one of the slips in Lot No. 2 to see if the centrifuging alone had had any effect on their germinating power, but these spores germinated practically 100 per cent. Cultures were made from these different lots from time to time as in the previous experiment, the results being given in the following table:

TABLE NO. 4.

EFFECT OF DRYING ON GERMINATION OF SPORES.

Lot. No.	Time Dried	Number of Plates	RESULTS
2....	0 days	1	100 per cent germination.
1....	1 day	1	Good germination, though not 100 per cent.
2....	1 day	1	Good germination, though not 100 per cent.
3....	1 day	1	Good germination, though not 100 per cent.
1....	3 days	1	Good germination.
2....	3 days	1	Good germination.
3....	3 days	1	Good germination.
1....	5 days	1	Good germination.
2....	5 days	1	Good germination.
3....	5 days	1	Good germination.
1....	7 days	1	Good germination.
2....	7 days	1	Good germination.
3....	7 days	1	Good germination.
1....	9 days	1	Good germination.
2....	9 days	1	Slight germination.
3....	9 days	1	Slight germination.
1....	11 days	1	No germination.
2....	11 days	2	Fair germination.
3....	11 days	2	Slight germination.
1....	13 days	2	Good germination. Hundreds of colonies.
2....	13 days	2	1 plate, no colonies; 1 plate, 14 colonies.
3....	13 days	2	Good germination.
1....	15 days	1	No germination.
2....	15 days	2	1 plate, 1 colony; 1 plate, hundreds of colonies.
3....	15 days	1	About 100 colonies in plate.
1....	17 days	1	Slight germination.
2....	17 days	1	30-40 colonies in plate.
3....	17 days	1	No germination.
1....	19 days	2	1 plate, no colonies; 1 plate, 18 colonies.
2....	19 days	1	2 colonies in plate.
3....	19 days	2	More than 100 colonies in each plate.
1....	22 days	2	1 plate, no colonies; 1 plate, 3 colonies.
2....	22 days	2	No germination.
3....	22 days	2	1 plate, no colonies; 1 plate, 200-300 colonies.

On the last day, all the cover slips which had been prepared were used, and the experiment was necessarily discontinued. While it is probable that there would have been some germination for a few days longer, most of the spores had lost their power to germinate at the end of twenty-two days. While some of the results are somewhat erratic, they show conclusively that it makes little difference to the spores whether they are imbedded in the mucilaginous substance or are freed from it from the standpoint of resistance to drying. From Hasselbring's results, it would have been expected that the centrifuged spores would have lost their power of germination in a day or so, but this was not the case. Perhaps, also, if Hasselbring had used nutrient media for the germination of the bitter rot spores, he would have obtained different results.

RELATION OF FUNGUS TO ACID AND ALKALI.

In a previous paper (6), attention has been called to the fact that the bean anthracnose is one member of this group that will not grow in a medium containing any great amount of acid. Most anthracnoses can be readily cultured by acidifying the medium in order to eliminate the bacteria, but the bean anthracnose is one of the forms that can not be cultured in this way. A medium that contains enough acid to eliminate the common forms of bacteria will also prevent the growth of the anthracnose. To test the amount of acidity this fungus would tolerate, bean agar was made, titrated, and brought up to different per cents of alkalinity and acidity. The per cents given are those of Fuller's scale. In the titrations, each time a sufficient amount of sodium hydrate was used to give the solution a good rose color. In the following table, I have compared the growth on the media with different acidity, with that of the cotton anthracnose, Glomerella gossypii. The results with the anthracnoses from the fig and pepper were very similar to those of the cotton form:

TABLE No. 5.

EFFECT OF ACIDITY AND ALKALINITY ON GROWTH.

Fungus.	Degree of Acidity or Alkalinity.							
	—16	—6	+5	+15	+20	+25	+38	+43
C. lindemuthianum	Fair	Good	Excellent	Good	Slight	None	None	None
G. gossypii	Good	Good	Excellent	Excellent	Excellent	Fair	Slight	None

The table does not show the amount of alkalinity the two fungi will tolerate, as they both grew fairly well on the medium with the highest percent of alkalinity, —16. But from the standpoint of acidity, the table shows a striking difference between the two fungi. The maximum acidity of the bean anthracnose is about +20, while the maximum for the cotton anthracnose is about +38. The optimum in both cases seemed to be about +5, though there was not a great deal of difference between —6 and +15.

RELATION OF FUNGUS TO TEMPERATURE.

The temperature factor is a very important one in regard to the bean anthracnose, especially in regard to its control. There are many instances on record where the spread and development of this disease has been suddenly checked, presumably from weather conditions. As a rule, these are said to be due to dry weather. But, after observations on the trouble for the past two years in a climate where the effects of the hot weather could be studied, it seems that the hot weather factor should be considered. While wet weather is essential for the spread of the disease, it is not essential for the development of the spots after the fungus has once gained entrance in the tissues of the bean plant.

In the northern states the temperature factor is not so important as in the south, on account of the lower mean temperature and the cooler nights. For only short spells during the growing season in the north is the weather so warm that it would be detrimental to fungi that can not tolerate a high temperature. It is probably due to this that the temperature

factor has been overlooked in the study of the disease. But little research work has been done on it in the south, and so the relation to temperature has not been observed.

The relation to temperature has been studied, under this investigation, both in the laboratory and in the field. On account of the lack of sufficient laboratory facilities, it has been impossible to work out the minimum, optimum, and maximum temperatures for growth, points which should be determined. But the data that has been obtained has shown conclusively that the bean anthracnose cannot tolerate a high temperature. The lower temperatures have always given a better growth and a better production of spores.

Perhaps the most conclusive evidence in this regard from the laboratory studies, was obtained in the difficulty in keeping the fungus alive in cultures through the summer. In the season of 1908, cultures of the fungus died out entirely, though the same precautions, with frequent transfers, were used, as with other fungi. I was not perfectly sure at the time that this might not have been due to carelessness in some way, so I tried again in 1909 to keep the fungus through the summer. The season was started with two different cultures. During the spring they grew very rapidly, producing an abundance of spores on bean pods or clover stems three days after the transfer. But when the hot weather came on in June, considerable difficulty was experienced. All during the summer frequent transfers were made, from one to three weeks apart. One culture died out in spite of anything that could be done. The culture did not become contaminated, as transfers gave absolutely no growth. However, with the other culture I was more fortunate. I was able to keep this alive, though at one time I thought that it was dead. The growth was extremely slow during the hot weather. For instance, transfers were made June 10. For several days there was no perceptible growth, and after fifty days—on July 30—the growth in these tubes (alfalfa stems) was less than one inch long. Transfers made on June 30, on sterilized bean pods, gave a very weak growth; twenty-five days later, the colonies on the pods being only six to eight millimeters in diameter. Many of the transfers that I made did die out, but from some I was able to keep up a weak growth. It was not until the cooler weather of September and October came on, that I

was able again to get a growth that was rapid and normal. Transfers made at this time gave a good, rapid growth, with an abundance of spores. The fungus had not changed during this period, as far as I could see. The growth looked identical with that of the previous spring, and inoculation experiments gave infection on young bean plants.

In the field, observations were made and experiments were conducted to test the effect of the temperature. While, under field conditions, there are other factors entering, as the rainfall and humidity, effect of micro-organisms of the soil, etc., the evidence seems conclusive that the temperature is a very important factor with this disease. The trouble was very bad during most of the month of May, but from the last two or three days in May on through the rest of the summer, there was no development of the trouble. The following table gives the daily maximum, minimum, and mean temperatures at Baton Rouge for a period during this time.

TABLE 6.

DAILY TEMPERATURE, BATON ROUGE, MAY 10 TO JUNE 20, 1909.

Day.	Max.	Min.	Mean.	Day.	Max.	Min.	Mean.
May 10.....	83	57	70	May 31.....	89	70	79.5
May 11.....	79	53	66	June 1.....	88	73	80.5
May 12.....	85	55	70	June 2.....	76	70	73
May 13.....	87	64	75.5	June 3.....	87	69	78
May 14.....	90	70	80	June 4.....	90	71	80.5
May 15.....	87	74	80.5	June 5.....	91	70	80.5
May 16.....	86	68	77	June 6.....	93	71	82
May 17.....	85	71	78	June 7.....	93	73	83
May 18.....	85	65	75	June 8.....	94	74	84
May 19.....	85	67	76	June 9.....	92	75	83.5
May 20.....	82	69	75.5	June 10.....	93	73	83
May 21.....	83	68	75.5	June 11.....	89	74	81.5
May 22.....	89	67	78	June 12.....	95	78	86.5
May 23.....	91	69	80	June 13.....	96	75	85.5
May 24.....	89	70	79.5	June 14.....	90	75	82.5
May 25.....	91	77	84	June 15.....	90	71	80.5
May 26.....	90	72	81	June 16.....	92	73	82.5
May 27.....	87	67	77	June 17.....	96	76	86
May 28.....	89	69	79	June 18.....	95	77	86
May 29.....	92	73	82.5	June 19.....	93	73	83
May 30.....	92	75	83.5	June 20.....	87	73	80

From June 20 until the latter part of September, the temperature, of course, averaged still higher. The daily mean from June 20 to September 20 varied from 79.5 to 91, with only one day below 80. The daily minimum for the same period ranged from 70 to 83, and only two days of that time was it as low as 70. Judging from the temperatures and the time when the anthracnose ceased to develop, it would seem that when the temperature reaches a daily mean of slightly above 80, with the minimum above 70, the critical temperature for the bean anthracnose has been reached. It would not be safe to draw a definite conclusion from this, however, on account of the many other factors which might influence the development of the disease.

The soil is also at a very high temperature during the summer months. Soil temperatures are not kept at any place in this region as far as I know, but in order to get some idea of how hot the soil gets, I made a few tests during one period of the summer. These could not possibly represent the maximum soil temperatures, for we had much hotter weather at other times during the summer. These temperatures were as follows:

TABLE 7.

SOIL TEMPERATURES.

Date. Hour.	Weather Condition.	Air Temp. Max. for Day.	Surface of Ground	2 in. Under Soft Earth	2 in. Under Packed Earth
July 29, 3:00 p. m.	Hot and sunshiny...	95	122	102	112
July 29, 4:30 p. m.	After slight rain....	95	96	97	98
July 30, 9:00 a. m.	Rain preceding night.	86	79	79	79
July 30, 3:00 p. m.	Cloudy, showers.....	86	88	87	87
July 31, 9:00 a. m.	Cloudy, ground wet..	89	84	82	82
Aug. 2, 9:00 a. m.	Hard rain preceding.	87	82	..	82

If the temperature factor is important in checking the disease, these high soil temperatures should work advantageously in checking the development of the disease on the cotyledons and young stems. In order to test this, a number of experiments were conducted.

On May 25, 1909, four rows of clean Wardwell's Kidney Wax seed were planted. The seed for two of the rows was treated with a suspension of anthracnose spores in water before planting. While ordinarily in the spring, in the field, or in the greenhouse in cooler weather, this would have given abundant infection, no spots containing anthracnose spores were found on the seedlings nor did any develop later on the plants.

A number of plots of diseased seed, ranging from 11 to 50 per cent spotted, were planted at various times during the summer to see if the disease would develop. These plantings did not consist of only a few seed, but were sufficient for a test. The smallest one consisted of four rows 110 feet long, and the largest about one-sixteenth of an acre. These were planted in June, July, August, and September. The young seedlings were examined carefully, but no spots containing developing anthracnose spores were found, nor did the plants develop anthracnose later. The lack of sufficient moisture was not the cause of lack of development, because, during most of the time, there was abundant rainfall.

On July 26, 1909, 100 spotted beans were carefully picked out. These all contained well developed anthracnose spots. These were planted in the garden at Baton Rouge. When the seedlings came up, these were pulled up and each one was examined microscopically for anthracnose spores, with the following results:

TABLE 8.

EFFECT OF TEMPERATURE ON ANTHRACNOSE DEVELOPMENT IN THE GROUND.

Date Examined	Total No. of Plants	Clean Seedlings	Spotted Seedlings	No. with Anthracnose	No. with Sterile Spots.	No. with Other Fungi*
July 31	43	26	17	2	14	1
August 3	46	26	20	2	6	12
August 5	1	1	..	..	..	..
Total	90	53	37	4	20	13

*The other fungi were Fusarium, Sterigmatocystis, Alternaria, and Penicillium.

The place where these beans were planted was in a low part of the garden, and, as there was considerable rainfall, the ground was wet during the experiment, thus decreasing the soil temperature to some extent. Yet, under these conditions, the small development of the anthracnose is remarkable.

Ninety of the 100 beans germinated, and of these 53 were clean. On the latter, it is evident that the disease did not extend deeper than the seed coats, and failed to develop further as the seed germinated. When the seed coats were shed, the cotyledons below were left clean. Thirty-seven of the young seedlings had spotted cotyledons, but on 20 of these the spots seemed to be perfectly sterile. The spots on the seeds of the latter evidently extended through the seed coats and into the cotyledons, before the seeds were placed in the ground. But further development of the disease was checked. The spots on the cotyledons of the young seedlings were merely smooth, sunken black spots, containing not a single anthracnose spore. These same spots, under cooler conditions, would have been covered with spores. Of the plants developing from the 100 diseased seed, only four of them had spots which contained anthracnose spores, and two of these had such a small number that it is questionable whether they were not some that were present on the seed when planted.

The effect of hot weather on the development of the disease on the cotyledons, is bound up with the question of the effect of other micro-organisms, a point which will be discussed later. The reason for the enumeration of the other fungi in the table will consequently be taken up at that time.

Other experiments in planting diseased seed and seed treated with anthracnose spores in the summer time in the laboratory have always failed to give a development of the fungus. Furthermore, fresh bean pods brought into the laboratory and sprayed with anthracnose spores in the hot weather have invariably failed to develop the disease, while others similarly sprayed in cooler weather produced spots in abundance after the normal period of incubation.

EFFECT OF OTHER MICRO-ORGANISMS.

Halsted has called attention to the fact that spotted seed rots much worse in the soil than clean seed, and, without doubt,

this has been noticed by others. But what this rot is due to, or what the factors are that favor or retard the development of the rot have not previously been investigated.

The relation of the rot organisms was one of the first points that appeared when the study of the bean anthracnose was commenced. Spotted seed often showed a low per cent of germination and, furthermore, as a rule the spots that were present on the young cotyledons contained spores of other fungi instead of the anthracnose spores. In the field plantings during each season, the seedlings developing from diseased seed have shown a high per cent to have the spots filled with some other fungus than the anthracnose. The most common fungus to be found in the spots was a species of Fusarium, though species of Penicillium, Alternaria, Aspergillus, and Sterigmatocystis were not uncommon. I was not able to determine the species of Fusarium, but it seemed to be the same pink one that is so common on various dead plants. The Fusarium, as a rule, developed so abundantly on the cotyledons that it was usually difficult to find a seedling on which there was a development of the anthracnose spores. Of course, some would escape the attack of the Fusarium, as shown by the fact that isolated plants would later develop the disease in abundance.

The bean anthracnose is a comparatively slow-growing organism, and it can not stand competition on dead material. Any of the common saprophytic fungi will readily run it out. As the tissue of the cotyledon is only in a semi-living state, it constitutes a medium upon which the saprophytic forms can grow. They obtain a foothold in the spot caused by the anthracnose and readily run the latter out. The Fusarium does not develop alone in the spots caused by the anthracnose, as it is often able to find entrance at other places; but the anthracnose spot furnishes the best conditions for its development. The Fusarium spores may be on the surface of the seed when it is planted, as I have frequently had it to develop when diseased seed was planted in sterilized soil, but more often it gains entrance to the seed from the soil.

The relation of the soil organisms to the bean anthracnose is a different problem in the south from what it is in the north. In the north, a large per cent of the saprophytic organisms are killed out or reduced during the winter, resulting in a rela-

tively small number of spores in the soil at planting time. This means that a relatively large per cent of the planted beans will not come in contact with them. However, in the south, where the winters are mild, the Fusarium and other fungi grow nearly the year around, and the soil is more or less thoroughly infected at planting time. However, even here the earliest plantings are freer from these forms than later plantings.

Having observed the rotting of the seed and the effects of the Fusarium on the young seedlings, some experiments were conducted in the greenhouse to get some more exact data on the question. The first experiment was started on February 1, 1909. Fifty ordinary six-inch pots were obtained and filled with good garden loam. These were numbered, divided in lots of five, and given the following treatment:

Pots 1-5. The soil was sterilized for one hour in the autoclave. Spotted bean seed was procured, washed for three minutes in a one-fourth saturated corrosive sublimate solution, rinsed in sterilized water, and then treated with a suspension of Fusarium spores in sterile water. The seeds, while still wet, were planted in the sterilized soil, five beans in a pot.

Pots 6-10. Soil sterilized for one hour. The spotted bean seed was treated with a suspension of Fusarium spores and planted while wet.

Pots 11-15. Soil sterilized for one hour. The spotted bean seed was washed for three minutes in a one-fourth saturated corrosive sublimate solution and planted without rinsing.

Pots 16-20. Soil not sterilized. The spotted seed was washed for three minute in a one-fourth saturated corrosive sublimate solution and planted.

Pots 21-25. Soil sterilized for one hour. The upper layer of the soil was treated with a suspension of Fusarium spores one day previous to planting. The spotted seed was planted without treatment.

Pots 26-30. Soil sterilized for one hour. The upper layer of soil was treated with a suspension of Fusarium spores at the time of planting. The spotted beans were planted without treatment.

Pots 31-35. Soil sterilized for one hour. The spotted seed was planted without treatment.

Pots 36-40. Soil not sterilized. The spotted seed was planted without treatment.

Pots 41-45. Soil sterilized for one hour. Healthy seed planted without treatment.

Pots 46-50. Soil sterilized for one hour. Healthy seed was wet with a suspension of Fusarium spores and planted.

In pots 1-40, five seeds were planted in each; in pots 41-50, six seeds in each. Davis White Wax seed was used in all the pots.

The spots on the young plants were examined microscopically. The results of the experiment are summarized in the following table:

TABLE 9.

EFFECT OF FUSARIUM AND SOIL STERILIZATION ON THE ANTHRACNOSE.

Pots No.	No. of Seed	No. of Plants	No. of Plants with Spots.	Spots with Fusarium*	Spots Entirely Fusarium
1- 5	25	23	16	2	0
6-10	25	23	19	7	4
11-15	25	19	10	0	0
16-20	25	15	12	0	0
21-25	25	22	20	15	7
26-30	25	21	12	4	2
31-35	25	23	19	0	0
36-40	25	5	2	0	0
41-45	30	28	0	0	0
46-50	30	28	0	0	0

In this experiment, all the spots that did not contain Fusarium spores contained an abundance of anthracnose spores.

There are two points which should be noticed in this table: First, notice the low per cent of germination of the diseased seed in non-sterilized soil (Pots 36-40), as compared to similar seed in sterilized soil (Pots 31-35). This shows that the anthracnose alone did not prevent the germination, but that there were organisms in the soil that could gain entrance to the diseased seed and rot them. In the seed washed in corrosive sub-

*Some of these spots contained both Fusarium and anthracnose spores.

limate and planted in non-sterilized soil (Pots 16-20), it seems that the poison prevented the entrance of some of the rot organisms, though the percentage of germination here is still much lower than in the sterilized soil. Second, notice the presence of the Fusarium in those which were treated with Fusarium spores, especially in pots 21-25, in which the Fusarium was allowed to get a start before the seeds were planted. It must be borne in mind also, that those spots which contain both anthracnose and Fusarium spores when they come through the ground, generally become completely overrun by the Fusarium later.

Other experiments gave similar results. In all cases where spotted seed was planted in soil treated with the Fusarium, many of the spots on the young seedlings contained nothing but Fusarium, while those planted in soil not treated produced plants with a good development of the anthracnose spores in most of the spots. These experiments, however, were disappointing in a way. While they showed that the Fusarium would eliminate a portion of the disease, it would not eliminate a sufficient amount of it to make seed or soil treatment practical.

The Fusarium seems to crowd out the anthracnose by its more rapid growth. It was thought at first that it might produce some toxic substance which would prevent the growth of the anthracnose, but some laboratory experiments seemed to disprove this. The Fusarium was grown for some time in bean bouillon. The liquid was then filtered through a Chamberland filter, and the sterile filtrate was added to other tubes of sterile media. The tubes were then inoculated with the anthracnose, with the result that it grew just as well in these as in the check tubes in which there was none of the filtrate.

The infection of germinating seed, cotyledons, or young stems, by means of anthracnose spores that may be on the seed or in the ground, is also greatly influenced by the rot organisms of the soil, both fungi and bacteria. To prove this, other pots were procured and filled with soil. Part of these were sterilized and part were not. The seed used for planting was all healthy, but it was treated with a suspension of anthracnose spores, some in a suspension in sterile water and the rest in potato bouillon. The following table gives the soil and seed treatment and the results of the experiment.

TABLE 10.

THE EFFECT OF SOIL STERILIZATION ON INFECTION AND SEVERITY OF THE ANTHRACNOSE.

Pots No.	Soil Treatment	Seed Treatment	No. of Seed	No. of Plants	No. Plants Spotted	No. Plants Killed*
51, 52	Sterilized 1 hr....	Spores, sterile water.....	20	18	18	18
53...	Sterilized ½ hr...	Spores, sterile water.....	10	10	10	10
54...	Not sterilized....	Spores, sterile water.....	10	5	4	1
55...	Sterilized 1 hr....	Spores in potato bouillon..	10	6	6	0
56, 57	Sterilized ½ hr...	Spores in potato bouillon..	20	18	16	11
58, 59	Not sterilized....	Spores in potato bouillon..	20	2	1	0
60...	Not sterilized....	Seed wounded; spores in water.....	10	3	3	1
61...	Sterilized ½ hr...	Seed wounded; spores in water.....	10	9	9	7
62...	Sterilized 1 hr....	Seed wounded; spores in water.....	10	9	9	7

An examination of the table will show that in the sterilized soil 70 seeds out of 80 (87.5 per cent) were able to germinate and push to the surface of the ground, while in the non-sterilized soil only 10 out of 40 (25 per cent) were able to do this. This shows again the effect of the various rot organisms in the non-sterilized soil. However, only 17 plants out of the 70 (24 per cent) were able to stand the effect of the anthracnose, the others rotting off at the surface of the ground; while in the non-sterilized soil eight out of the ten plants lived (80 per cent), only two rotting off. If the seed is infected with anthracnose spores, and these are not in competition with other micro-organisms, the anthracnose is able to cause a very high mortality among the small seedlings. However, if the rot organisms are in the soil, these will greatly reduce the severity of the anthracnose attack. Of course, these will prohibit the germination of many seed that are infected with the anthracnose, but this is a benefit from the standpoint of the control of the trouble. Plate IX shows two pots from this experiment, one with the sterilized and the other with the non-sterilized soil. All the plants are dead in the sterilized soil, with all parts covered with anthrac-

*A large number of plants, especially in the sterilized soil, rotted down just after germination with the anthracnose.

nose spores, while the plants in the non-sterilized soil have a much better appearance. These also contain small anthracnose spots, but the severity of the attack has been greatly lessened.

From the standpoint of the bean raiser, the effect of the soil organisms is very important. While the disease is not entirely eliminated in the field, it is reduced from a condition that would have been general, if all the spotted seed planted had grown, to a localized one. With the disease reduced to a localized condition, it can usually be kept in check with the precautions which will be discussed later under "Control."

RELATION OF THE FUNGUS TO OTHER ANTHRACNOSES.

Much has been written in regard to the limits of species among the anthracnoses. Formerly, to a large extent, anthracnoses were determined by the host; that is, it was thought that the forms found on different hosts were distinct. But of late years, since these have been studied carefully, the ideas concerning the species have been modified considerably. At present, some go so far as to say that we have but one or two species of these forms that are connected with the Glomerella perfect stage. Shear and Wood (29) would place all of these forms, with perhaps the exception of the cotton anthracnose, in one species, with only a varietal difference at the most between them. As long as we do not know what a species is, and we hardly have two botanists who have the same idea in regard to the limits of species, the final disposition of forms such as we have in the group of anthracnoses, is indeed a question. We know that there are differences between some of the anthracnoses, differences that are constant; furthermore, we know that some of the anthracnoses are peculiar to some host plants and cannot be transferred to others. Whether these differences are specific differences is a question, but it is absolutely necessary that they should be designated in some manner. It will not do to call them all Glomerella rufomaculans; and the use of a varietal name in the form of a trinomial is, to say the least, very inconvenient. For me it seems better to call the different forms different species until we have better ideas of the limits of species in this group. Of course, if a form will grow and does

grow normally on a number of different hosts, as the form on apple is known to do, it can have but one name. But if a form is peculiar to a single host and will not grow on others, it should have a different name from any anthracnoses that might be found on these other hosts. The bean anthracnose seems to be such a form, and, consequently, is considered as a distinct species in this bulletin. The reasons for this are the differences in cultural characteristics which have been discussed, and the results of cross inoculation experiments, which will be described later.

Halsted (15) seems to have been the first one to try cross inoculation experiments with the bean anthracnose. He inoculated a citron with the anthracnoses from the bean and watermelon. According to his statement, the spots caused by the two fungi grew together, and it was impossible to tell the boundary between them. From this experiment he claimed that the bean and watermelon anthracnoses were the same, calling them both Colletotrichum lagenarium. As far as I know, the experiment has never been duplicated by others.

C. O. Smith (31) tried some careful experiments in inoculating the bean anthracnose on the watermelon, and the watermelon anthracnose on the bean, but with no infection in either case. Consequently he claimed that the two fungi were distinct. Miss Stoneman (33), from a study of cultural characters, arrived at the same conclusion. Sheldon (30) also, was unable to infect the bean by using the watermelon anthracnose.

The cross inoculations which have been tried during this investigation have been with the forms from the bean, cotton, fig, pepper, and rose. The work has included the inoculation of the bean anthracnose on hosts other than the bean, and the inoculation of anthracnoses from other hosts on to the bean. The inoculations were all made in the greenhouse, where the conditions could be controlled. The results of these inoculations are briefly given in the following paragraphs:

Experiment March 2, 1909. Sprayed bean anthracnose spores on young bush bean plants, alfalfa, and cotton plants, protected by bell jars, with the following results:

On bush beans—abundant infection.

On alfalfa—no infection.

On cotton—no infection.

Experiment March 22, 1909. Sprayed bean anthracnose spores on young bush beans, pole beans, Lima beans, and garden peas, with the following results:

Bush beans—abundant infection.

Lima beans (variety, Small White Pole,)—several small spots developed.

Pole beans (variety, Nox All,)—no infection.

Garden peas—no infection. On some of the garden peas the wax was rubbed off the leaves so that the water containing spores would adhere better, but with no better results.

Experiment April 5, 1909. Sprayed the bean anthracnose on young bush bean, pole bean, Lima bean, and cucumber plants, with the results given in the table below. The pole beans were of the variety Nox All, and the Lima beans the Small White Pole Lima.

TABLE 11.

INFECTION OF PLANTS WITH BEAN ANTHRACNOSE.

Plants used.	April 10.	April 11.	April 12.	April 17.
Bush beans	Slight infect.	Abundant infec.	Abundant infec.	Abundant infec.
Pole beans	No infection	No infection	No infection	Found one spot
Lima beans	No infection	No infection	Slight infection	Slight infection
Cucumber	No infection	No infection	No infection	No infection

Other attempts to inoculate cucumbers at various times, using leaves, stems and fruits, were unsuccessful.

Experiment March 22, 1909. Sprayed young bush bean plants with the bean, fig and pepper anthracnoses, with the following results:

Bean anthracnose gave abundant infection.

Fig anthracnose gave no infection.

Pepper anthracnose gave no infection.

Experiment May 4, 1908. Placed freshly picked bean pods in moist chambers and sprayed on them cultures from the bean and from the rose, with the following results:

Bean anthracnose gave abundant infection.

Rose anthracnose gave no infection.

Experiment February 10, 1909. Planted healthy Davis White Wax beans, which were wet with suspensions of spores of the

bean, fig and cotton anthracnoses, in pots in the greenhouse. The amount of germination, and the amount of disease on the young plants is given in the following table:

TABLE 12.

EFFECT OF DIFFERENT ANTHRACNOSES ON GERMINATING SEED.

Anthracnose	No. of Seed	No. of Plants	No. with Spotted Cotyledons at First	No. Spotted Feb. 23.	No. Spotted Feb. 25
Bean	25	21	9	19	20
Cotton	25	24	1	0	0
Fig	25	18	1	1	0

The fig anthracnose, especially, seemed to be able to grow on the substance of the bean seed, for several of the beans that rotted in the ground without germination were covered with the spores of this fungus. Both the cotton and the fig anthracnoses were able to attack the semi-living cotyledons of the germinating beans, and in one case, with each, cotyledons spotted with these were carried above the ground. These spots, however, did not look at all typical of bean anthracnose spots, though they contained spores in abundance. These spots did not develop further; the cotyledons finally fell off and the plants were left clean. But with the bean anthracnose there was a good development of the disease on the cotyledons and, later, abundant development on the stems and leaves.

This experiment was repeated with the bean and fig anthracnoses, using sterilized soil instead of the non-sterilized, as used in the previous one. In this case, practically all the young plants growing from the seed treated with the bean anthracnose were spotted and the later development of the disease on the leaves and stems was abundant. The plants that grew from the seed treated with the fig anthracnose had no definite spots, but several had irregular decayed areas containing anthracnose spores. But when the cotyledons fell off, the plants were left clean.

A similar experiment was also tried using the bean and rose anthracnose with very similar results. Plate XI, figure 1,

shows bean cotyledons affected with the rose anthracnose. With this anthracnose, as with the fig, the bean plants were left clean when the cotyledons fell off.

From the experiments described above, and from the work of other investigators, there seems to be no doubt that the bean anthracnose is a specialized form among the anthracnoses, at least from a physiological standpoint. It is practically confined to the host, Phaseolus vulgaris, though it will take to a slight extent on at least some varieties of the Lima bean, Phaseolus lunatus. It has also been reported on the Scarlet Runner, Phaseolus multiflorus, by Massee (23). It has also been reported on the cowpea, Vigna catjeng, by Chester (4), but the proof of the identity of the form is far from conclusive. The bean anthracnose is certainly distinct from the fruit rot anthracnoses.

CONTROL.

A large part of the work that has been done by workers in plant pathology on the bean anthracnose has been along the line of control. This has included work on spraying, seed treatment, the use of clean seed, the eradication of diseased plants, proper cultivation, the use of resistant varieties, etc. In the light of our present knowledge of the disease, a discussion of these different factors which tend to increase or decrease the severity of the disease will not be out of place.

Natural Factors.

Under Louisiana conditions, the effect of the natural factors of control—heat and micro-organisms—is very important. These have both been discussed in earlier pages of this bulletin, but little has been said in regard to their relation to control. Under ordinary conditions, these decrease the severity of the disease considerably. But these two factors are not made as much use of as they should be. Beans are generally grown at the time when these factors are not especially important. To make beans profitable, they must be planted as soon as there is little danger of frost, this being during the first half of March. This brings the beans into marketable shape during the last of April and the first of May. This is just the time when the natural

factors are of the least importance. The cool and wet weather of the spring is most favorable for the development of the anthracnose, and by planting so early in the spring, there is not so much chance for the soil organisms to eliminate the disease on the young seedlings. The question is, how can we utilize these natural factors to better advantage? This can be done by growing some beans during the warmer part of the year and saving the seed for planting the following spring. It has been demonstrated, especially by the work of Whetzel (37), that the disease can be controlled by the use of clean seed. If clean seed is used and the disease is not introduced into the field later, there will be no development of the anthracnose. Plantings of clean seed on the Experiment Station grounds at Baton Rouge gave an absolutely clean crop, though there were badly diseased plots less than 100 yards distant. Now, if we can use the natural factors of control, in raising beans that can be used for seed purposes, we have progressed towards the solution of the problem. In Louisiana Bulletin 116, the planting of a small plot in the fall for seed purposes was advised. During two seasons' work in Louisiana, I have not seen a spot on a bean due to the anthracnose later in the season than June. There is often some spotting due to the blight, but the anthracnose is lacking. According to the statements of some bean growers, it does appear occasionally in the fall, but not usually. And it is more than possible that these truckers had confused the anthracnose with the blight.

There is, however, liable to be some difficulty in raising a crop of beans in the fall. Beans are accustomed to a cool climate and will not set pods during the hot summer months. In order to produce a crop in the fall, it is necessary to plant the beans late enough in the season, so that the weather will be fairly moderate at blooming time. This will allow the beans to be planted in the warm weather, thus allowing the heat and soil organisms to kill out the disease before the cool weather begins. If the frosts do not come too early, it is possible to raise seed in the fall. However, there is always a chance of the frosts killing the plants before the pods mature. However, some seed has been raised during the past two years in this way.

During 1909 experiments were conducted at Baton Rouge to test the possibility of raising fall seed. Plots were planted at intervals of two weeks during the summer, beginning about the first of July. All of the early plantings were, of course, failures on account of the hot weather. No pods set and finally the plants died. The planting made July 29, was the first that set any pods, and this was too early for the best results. However, the summer of 1909 was exceptionally hot and the warm weather continued longer than usual in the fall. It is possible that in the more sandy parts of the State, as in Tangipahoa parish, early plantings would do better than at Baton Rouge. Not alone were we handicapped by the hot weather during 1909, but also by the hurricane of September 20. This storm nearly destroyed all the plants that we had on the station grounds, breaking many off, defoliating many, and washing the soil away from the rest, so that most of them had the roots exposed. The recovery of the plants took place very slowly. Consequently we did not gather a great deal of seed, but what we did gather was perfectly free from anthracnose. Not an anthracnose spot developed through the summer or fall in any of these plots, though seed as bad as 50 per cent spotted were used in some of them.

Experiments in growing fall seed will be continued next season. Also we intend to try planting beans later in the spring, so that they will mature in the summer, and see if it is not possible to eliminate the disease in the seed in this manner. A few preliminary experiments in this have given good results.

Practically all of the beans that are raised in Louisiana are grown from northern grown seed. Truckers, as a rule, seem to think that southern grown seed will not be as productive or early as the northern seed. However, from experiments conducted at the Experimental Station a few years back, there seems to be no ground for this belief (34). This being the case, it would seem that the home production of seed would be advisable for the Louisiana trucker. Even if he does not try to grow his seed during the warm weather and saves his seed from his spring crop, he will have cleaner seed than he usually gets from the north.

CULTIVATION.

The effect of working the plants while wet with rain or dew has been discussed in Bulletin 116, and the details need not be repeated. It is enough to say that in a field where the disease was at first localized in narrow strips across the field and worked only while the plants were dry had 8.8 per cent more clean pods than a similar field that was worked while the plants were wet. It is an old saying in bean growing sections that cultivation of the plants while they are wet will spot them; and it is a saying that is well founded.

The cultivation of beans should be thorough, as thorough as with any other crop. It is often the case that the cultivation of this crop is slighted, especially if there is other work to do at the time. Cultivation will not only increase the yield, but will also hasten the formation of the beans, thus affording a shorter time for the beans to become infected with the disease.

FERTILIZATION.

The effect of the fertilizers on the bean anthracnose has not been studied enough to furnish any reliable data. But with this, as with cultivation, anything that tends to force the plants will help in controlling the disease. The effect of barnyard manure on the development of the plants and their resistance to the heat of summer, was observed in some of the plots described above. Two plots were planted on July 29. One of these had a good treatment of stable manure during the spring, while the other plot had no fertilizer. The unfertilized plot died out very badly during the summer, the plants setting no pods. The plants in the fertilized plot died somewhat, but they were much healthier than in the other plot and set a fair crop of pods. In order to obtain a good crop of beans in the fall, it seems very essential to have the ground well enriched with stable manure. Just what the action of the stable manure was, is a question. It may have helped the physical condition of the soil, by increasing the amount of humus in it. This would tend to keep down the temperature.

SEED TREATMENT.

Considerable work has been done on seed treatment, but no treatment has been devised which seems to be practical. Beach,

Halsted and others have treated the seed with various chemicals, as Bordeaux mixture, other copper compounds, mercuric chloride, iron sulphate, lysol, etc., but with the general result that the disease was not entirely killed out with chemicals weak enough to cause no harm to the germinating power of the seed. Treatment with hot water, as described by Beach, seems to have given the best results. This treatment has also been recommended for killing the blight bacteria on the seed.

Some preliminary work on seed treatment was commenced during the summer of 1909. First, healthy seed was treated with water at various degrees of temperatures for definite periods to see how much the seed itself would stand. After the seed was treated, it was planted in the field. Fifty seed were used in each treatment. The treatment and the results were as follows:

TABLE 13.

EFFECT OF SEED TREATMENT ON GERMINATION.

No.	Treatment of Seed.	Germination, per cent.
1.	Seed untreated	96%
2.	Bordeaux mxture 5 min., solution drying on seed.	94
3.	In water at 50° C. for ten min	96
4.	In water at 60° C. for 5 min	82
5.	In water at 70° C. for 1 min	90
6.	In water at 80° C. for 1 min	20
7.	In water heated gradually to 50° C., time necessary 10 min	88
8.	In water heated gradually to 60° C., time necessary 15 min	58
9.	In water heated gradually to 70° C., time necessary 20 min	0
10.	In water heated gradually to 50° C., and held at that point 5 min	86

From this it would seem that seed treated in water at 50° C. (122° F.) for ten to fifteen minutes has little or no effect on germination. Furthermore, this short treatment is not sufficient to cause the seed coats of the beans to loosen. If the seed slips its coat, it is worthless for planting.

Having some idea of the resistance of the seed itself to treatment, some diseased seed was treated and planted in pots. As the time when this planting was done was in the summer,

most of the pots were placed in a cool basement, where the effect of the high temperature would be eliminated as far as possible. However, a few were left in the greenhouse, where it was warm. The treatment of seed and soil, the location of the plants, and the results are given in Table 14. The numbers under Seed Treatment refer to the numbers in Table 13.

TABLE 14.

EFFECT OF SEED TREATMENT ON BEAN ANTHRACNOSE.

No. of Seed	No. of Plants.	Soil Treatment.	Location.	Seed Treatment.	Spotted Plants.	No. Anthracnose.	No. Fusarium.	No. Other Fungi.
20	16	Sterilized	Cool room	1	6	4	2	0
20	15	Sterilized	Cool room	2	9	5	1	3
20	15	Sterilized	Cool room	10	13	1	10	2
20	11	Sterilized	Cool room	4	11	0	7	4
20	13	Sterilized	Cool room	5	10	0	6	4
20	17	Sterilized	Warm room	1	13	0	3	10
20	8	Not sterilized	Cool room	1	5	3	0	2
20	16	Not sterilized	Warm room	1	12	2*	2	8

This table shows that the plants that grew from the seed treated with hot water had a smaller per cent diseased than from the untreated seed. While this shows that this treatment may be beneficial, it was not conducted on a large enough scale to be conclusive. This table also shows again the effect of hot weather in reducing the anthracnose.

Considering that a considerable per cent of the anthracnose on diseased seed under Louisiana conditions will be eliminated by the natural factors, it may be that seed treatment with hot water will help materially in decreasing the remainder. Barlow (18) has also pointed out that the blight bacteria may be killed on the seed by the hot water treatment. If this is the case, seed treatment may become an important factor in Louisiana in the control of bean diseases. But at present more data is necessary.

*Only a very few scattering spores and these very much shrunken and vacuolate. Without doubt these were some of the spores that were on the seed before it was planted.

SPRAYING.

Spraying has been advocated by many, and is still being advocated by some, as a remedy for bean diseases. Halsted has perhaps carried out the most work in this, but the evidence he has obtained is somewhat conflicting. Whetzel (37) from his work in New York, claims that spraying with any spray machinery we have at present is not satisfactory in controlling the bean anthracnose.

No experiments to prevent the anthracnose in Louisiana by spraying have been tried. Some experiments to prevent the blight and to decrease the attack of insects were tried in the summer of 1909. Bordeaux mixture and arsenate of lead were used. On account of the very frequent rains in this region, it is necessary to spray on an average of about once a week. This spraying not only decreased the blight, but also protected the plants to some extent from the effects of the hot weather. The unsprayed plots in many cases died early, while the sprayed plots held their green color for some weeks later.

We can hardly expect to control the anthracnose in Louisiana by spraying. Even in dryer regions it has proven to be unsatisfactory.

RESISTANT VARIETIES.

No variety of bush beans, as far as I know, is entirely resistant to the anthracnose, and there is no reliable data in regard to the comparative resistance of the different varieties. Several investigators have grown different varieties side by side and then determined the amount of anthracnose on the different ones. But experiments of this kind are of little value. The mere fact that one variety spots badly under these circumstances while another spots but slightly, shows but very little. The chances are that the seed of the variety that spotted badly were much more diseased to begin with than the other. The only way to get reliable data on this point would be to give each variety a thorough spraying with a suspension of spores. All the wax varieties that I have worked with, including Wardwell's Kidney Wax, Davis' White Wax, Refugee Wax, and Challenge Black Wax, have been very susceptible. The small white bean, commonly called the Navy bean, is also very susceptible. The Valentine, a green podded variety, is slightly

more resistant. The leaves and stems of the Nox All, a pole variety, have shown highly resistant qualities. No inoculations were tried on the pods of this variety. Hodson's Wax is getting a reputation in the trucking districts of being more or less resistant to this disease, but it certainly is not entirely resistant, as I have observed the anthracnose on this variety at Ponchatoula. No inoculations have been tried on this variety, and it is questionable whether the freedom of the disease is not more or less due to cleaner seed.

SOURCE OF SEED FOR PLANTING.

Practically all the seed used for planting in Louisiana is northern grown seed, coming principally from Michigan and Colorado. The greater part of it comes from Michigan, though of late years Colorado seed is becoming popular. The Michigan seed comes from a state where anthracnose is very severe, and, as a result, the per cent of diseased seed in that obtained from that region is often large. In Colorado, the seed is grown in the irrigated regions, and the growers claim that the anthracnose is rare. During the past season, at least, plants grown from Colorado seed in Louisiana were freer from the anthracnose than most other seed. However, plants grown from Colorado seed were much more severely affected with the blight than the other seed, so it is a question whether there was anything gained by the use of this seed.

FURTHER WORK ON BEAN TROUBLES.

It is expected that the work on bean troubles will be continued at the Louisiana Station, being principally along the following lines:

1. Experiments on raising home-grown seed in the late spring and fall in order to get clean seed for planting.
2. Experiments to determine whether seed grown in the south is inferior in earliness or yield to northern-grown seed.
3. To test the efficiency of sprays as a control for the blight.
4. To test the efficiency of the hot water treatment of seed in eliminating the anthracnose and blight.
5. Further experiments in transferring the bean anthracnose to other hosts, and other anthracnoses to the bean.

SUMMARY.

Briefly the results of this investigation may be summed up as follows:

1. The bean anthracnose is a disease which causes a spotting of all parts of the bean plant above ground.

2. The disease is practically confined to varieties of Phaseolus vulgaris, though plants of at least some varieties of Limas will become spotted.

3. The period of incubation of the fungus is from four and one-half to six days, if the conditions for growth are favorable.

4. The disease is carried over from season to season in the seed, by means of resting mycelium and spores. The spores are present either on the surface of the diseased spots, or between the cotyledons, or in special enclosed pycnidia in the tissue of the bean.

5. Spores on the seed and in the seed are capable of retaining their virulence until planting time.

6. There is little difference in the viability of the spores whether they are dried down in the mucilaginous matrix which surrounds them, or whether they are freed from this.

7. The method of spore germination depends upon the medium, the number of spores in the medium, and the age of the spores.

8. The fungus will not stand a high per cent of acidity in the medium, thus differing from many other anthracnoses.

9. The fungus will not tolerate a continued high temperature, and consequently is killed out during the summer months in Louisiana.

10. Other micro-organisms in the soil, especially a species of Fusarium, easily gain entrance to the diseased seed and either rot the seed entirely or run out the anthracnose in the spots.

11. The natural factors, heat and micro-organisms, are very important in the control of the disease under Louisiana conditions, and it is thought that the proper use of these in growing seed for planting will greatly decrease the trouble.

BIBLIOGRAPHY.

The following bibliography does not include all references to this disease; only those mentioned in the text and a few others of importance:

1. Atkinson, G. F., Some observations on the development of Colletotrichum lindemuthianum in artificial cultures. Bot. Gaz. *20*:305-312. 1895.
2. Beach, S. A., Some bean diseases. N. Y. Agr. Exp. Sta. Bull. *48*. 1892.
3. Briosi and Cavara, Colletotrichum lindemuthianum (Sacc. and Magn.) Br. and Cav. I. Funghi parasiti della pianti coltivate ed utile, No. 50, Pavia Italy. 1889.
4. Chester, F. D., Diseased cowpea seed. Del. Agr. Exp. Sta. 6th Report, *1893*:110-111. 1894.
5. Craig, J. (Treatment of Bean Anthracnose). Can. Expt. Farms Rep. *1897*:110-120.
6. Edgerton, C. W., The physiology and development of some anthracnoses. Bot. Gaz. *45*:367-408. 1908.
7. ———, Preliminary report on the anthracnose or pod spot disease of beans. La. Agr. Exp. Sta. Bull. *116*. 1909.
8. Fawcett, H. S., Bean anthracnose. Fla. Agr. Exp. Sta. Rep. *1906*:XXI-XXII. 1907.
9. Ferris, E. B. (Bean anthracnose). Miss. Exp. Sta. Bull. *101*:9. 1907.
10. Frank, B., Ueber einige neue und weniger bekannte Pflanzenkrankheiten. Ber. der deutschen botan. Gesell. *1*: 31-34. 1883.
11. Fulton, H. R., Diseases of pepper and beans. La. Exp. Sta. Bull. *101*:1-21. 1908.
12. Gain, E. (Studies on bean anthracnose). Compt. Rend. Acad. Sci., Paris, *127*:200-204. 1898.
13. Galloway, B. T., Anthracnose of the bean. U. S. Dept. of Agr., Div. of Botany, Bull *8*. 1889.
14. Halsted, B. D. (Bean anthracnose). N. J. Exp. Sta. Rep. *1891*:284-288. 1892.
15. ———, Identity of anthracnose of bean and watermelon. N. J. Exp. Sta. Rep. *1893*:347-352. 1894.
16. ——— (Bean anthracnose). N. J. Agr. Exp. Sta.
Rep. *1894*:294-302, 335-336. 1895.
Rep. *1895*:283-292. 1896.
Rep. *1896*:328-332, 356, 363, 368, 389. 1897.
Rep. *1897*:309-314, 351-354. 1898.
Rep. *1898*:309-312. 1899.
Rep. *1899*:379-382. 1900.

Rep. *1900*:419-421. 1901.
Rep. *1901*:389-390. 1902.
Rep. *1903*:485. 1904.

17. ———, Bean diseases and their remedies. N. J. Exp. Sta. Bull. *151*. 1901.
18. Harrison, F. C., and Barlow, B., Some bacterial diseases of plants prevalent in Ontario. Ont. Agr. College Bull. *136*. 1904.
19. Harvey, F. L., Bean anthracnose. Maine Exp. Sta. Rep. *1893*:152-153.
20. Hasselbring, H., The appressoria of the anthracnoses. Bot. Gaz. *42*:135-142. 1906.
21. McCarthy, G. (Gloeosporium on cowpeas). N. C. Exp. Sta. Bull. *98*:151.
22. ——— (Bean anthracnose). N. C. Exp. Sta. Bull. *84*:14. 1892.
23. Massee, G., French bean canker. Gard. Chrn. 3 ser., *23*, No. 594:293. 1898.
24. Rolfs, P. H., Anthracnose of the bean. Fla. Exp. Sta. Rep. *1896*:48-49.
25. Rolfs, F. M., Bean Anthracnose. Fla. Exp. Sta. Rep. *1905*:30.
26. Saccardo and Magnus, Gloeosporium lindemuthianum n. s. Michelia *I*:129. 1878.
27. Scribner, F. L., Anthracnose of the bean. U. S. Dep't Agr., Sec. Veg. Path., Rep. *1887*:361-364.
28. ———, Anthracnose of the bean. Orchard and Garden *XI*:193-194. 1889.
29. Shear, C. L., and Wood, Anna K., Ascogenous forms of Gloeosporium and Colletotrichum. Bot. Gaz. *43*:259-266. 1907.
30. Sheldon, J. L., Diseases of melons and cucumbers. West Va. Exp. Sta. Bull. *94*. 1904.
31. Smith, C. O., Bean anthracnose. Del. Exp. Sta. Bull. *63*:24-28. 1904.
32. Stone, J. L., Commercial bean growing in New York. Cornell Exp. Sta. Bull. *210*. 1903.
33. Stoneman, Bertha, A comparative study of the development of some anthracnoses. Bot. Gaz. *26*:69-120. 1898.

34. Stubbs, W. C., Burnette, F. H., and Watson, Eugene, Home grown vs. purchased seed. La. Exp. Sta. Bull. *68*. 1902.
35. Viala, P., and Pacottet, P. (Investigations on grape anthracnose). Rev. Vit. *24*:433-439, 489-496, 517-523, 573-580, 601-608, 657-663. 1905. *25*:89-91, 341-347, 369-375. 1906.
36. Whetzel, H. H., Some diseases of beans. Cornell Exp. Sta. Bull. *239*. 1906.
37. ———, Bean Anthracnose. Cornell Exp. Sta. Bull. *255*. 1908.

EXPLANATION OF PLATES.

PLATE I. Colletotrichum lindemuthianum on pods of cultivated beans. Spots produced by inoculation.

PLATE II. Colletotrichum lindemuthianum, showing spots on under side of leaf and on petiole.

PLATE III. Colletotrichum lindemuthianum, showing spots on cotyledons.

PLATE IV. Colletotrichum lindemuthianum, showing spots on the stems.

PLATE V. Colletotrichum lindemuthianum. Fig. 1: The crumpling of the leaves due to the fungus. Fig. 2: Cross section of diseased pods.

PLATE VI. Colletotrichum lindemuthianum, showing very old spots on the stems.

PLATE VII. Colletotrichum lindemuthianum. Fig. 1: The spots on the seed. Fig. 2: Cross section of a spot on a pod, showing localized condition and the shrunken tissue.

PLATE VIII. Effect of inoculation on young bean plants. The plants on the left were sprayed with a suspension of spores, the photograph being taken the second day after the spots appeared. The plants on the right were not inoculated.

PLATE IX. Effect of sterilized and non-sterilized soil on the development of the anthracnose. In pot on left, the soil was sterilized and seed wet with a suspension of anthracnose spores. All plants have rotted off with the disease. In pot on the right, the soil was not sterilized, otherwise the treatment was the same. These plants show a much weaker infection.

PLATE X. Effect of non-sterilized soil on spotted and clean seed. The pot on the left was planted with clean seed, the one on the right with spotted seed.

Plate XI. Fig. 1: Shows spot on a cotyledon produced by the rose anthracnose. Fig. 2: Shows the development of the Fusarium in the anthracnose spots on the cotyledons.

Plate XII. Successive stages in the development of the acervulus of the bean anthracnose on bean pods. Fig. 1: A collection of a few mycelial threads in the epidermal and sub-epidermal cells. Fig. 2: The mycelial threads shown in the previous figure have given rise to a number of hyphal threads which lie nearly perpendicular to the surface of the bean. The increased growth of these raise the cuticle. Figs. 3 and 4: Later stages of the same. The hyphal threads which become the conidiophores, increase in number. Fig. 5: Later stage. The beginning of spore formation under the still unbroken but raised cuticle. Fig. 6: Still later stage, showing a large acervulus, spore formation, and the formation of a stroma at the base.

Plate XIII. Figs. 1 and 3: Later stages in the development of the acervulus. Fig. 1: The ruptured cuticle still keeps the exudation of spores confined to a small opening. Fig. 3: The cuticle is entirely thrown aside. Figs. 2 and 4: Cross section of a diseased spot on the seed. Fig. 2 shows two pycnidia filled with spores, buried in the tissue of the bean. Fig. 4 shows one of these pycnidia much enlarged, showing conidiophores and spores.

Plate XIV. Spore and mycelial characters. Fig. 1: Normal appearance of spores. Fig. 2: Vacuolate spores, first stage of deterioration. Fig. 3: Later stage than preceding. Spore contents broken away from the spore wall and collected in masses in the center. Spores not viable. Fig. 4: Normal germination of spores in nutrient media. Fig. 5: First stage in germination of spores that are too much crowded in the nutrient media. Fig. 6: Later stage of the same. Fig. 7: Still later stage of the same. Shows the formation of spores on the much swollen spore. Fig. 8: The germination of spores that have nearly lost their viability, in nutrient media. Fig. 9: The formation of conidia. Fig. 10: Appressoria that developed in water. Fig. 11: Appressoria that developed in cultures. Fig. 12. Anastamosing of mycelium from germinating spores. Fig. 13: The large, colored, thick walled threads that make up the crust or stroma in the old cultures. Fig. 14: An earlier stage of the preceding.

PLATE I.

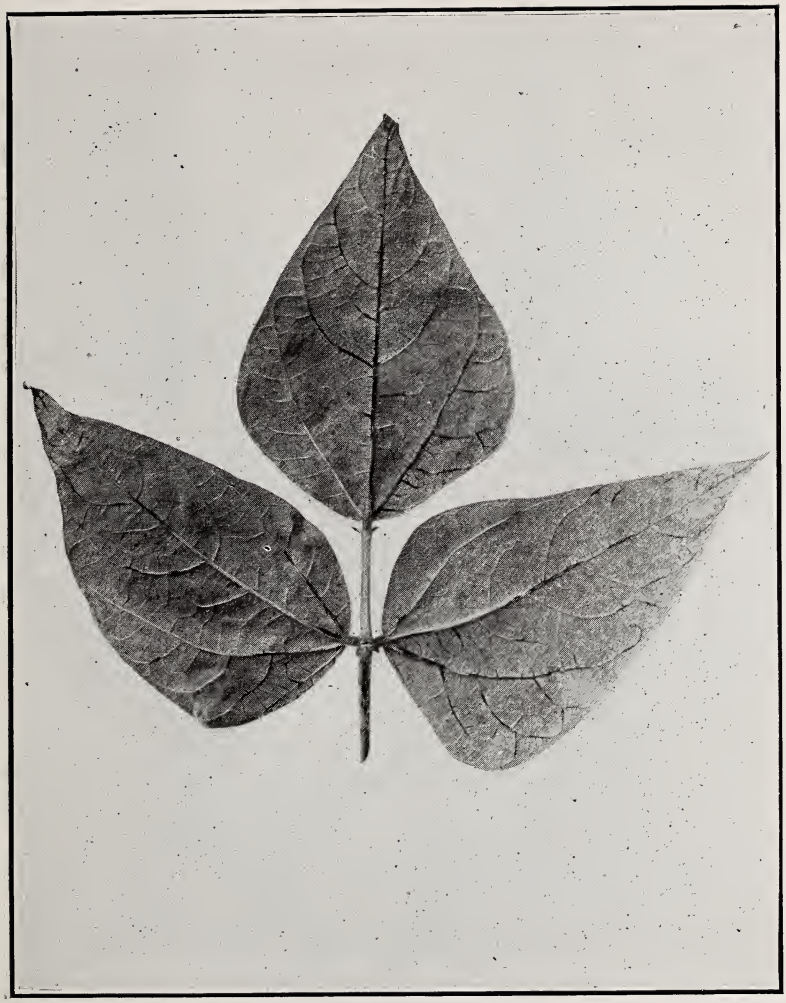
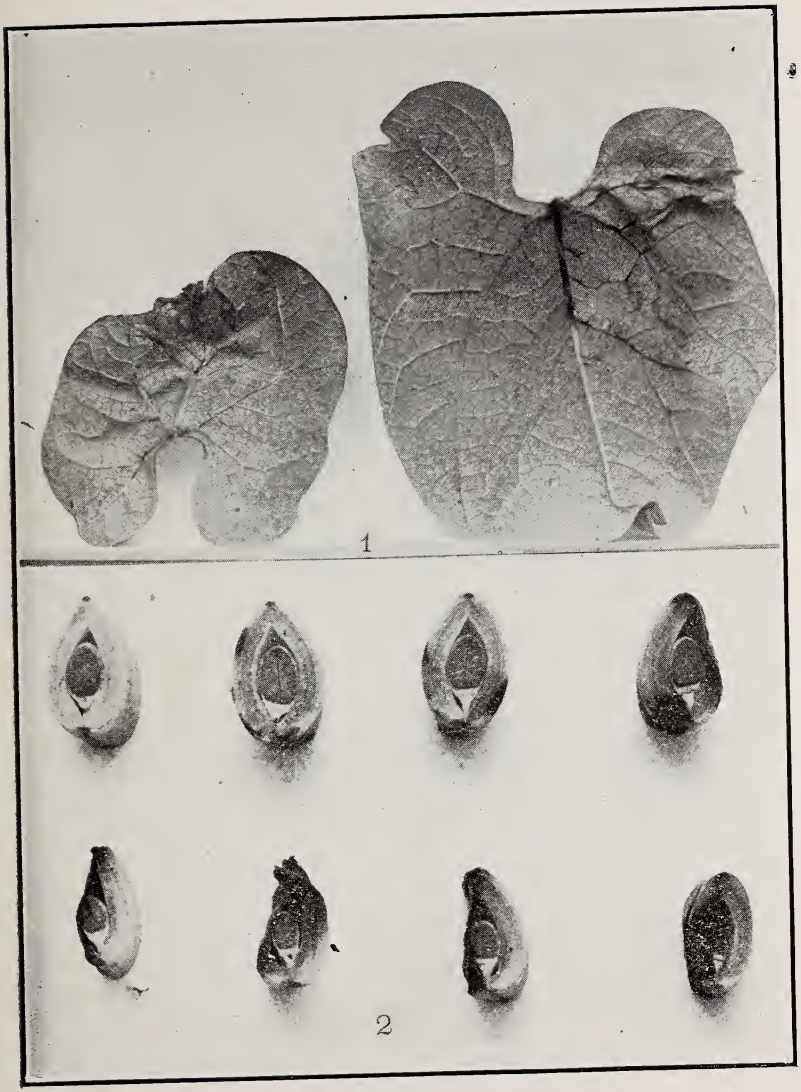


PLATE II.

PLATE III.

PLATE IV.



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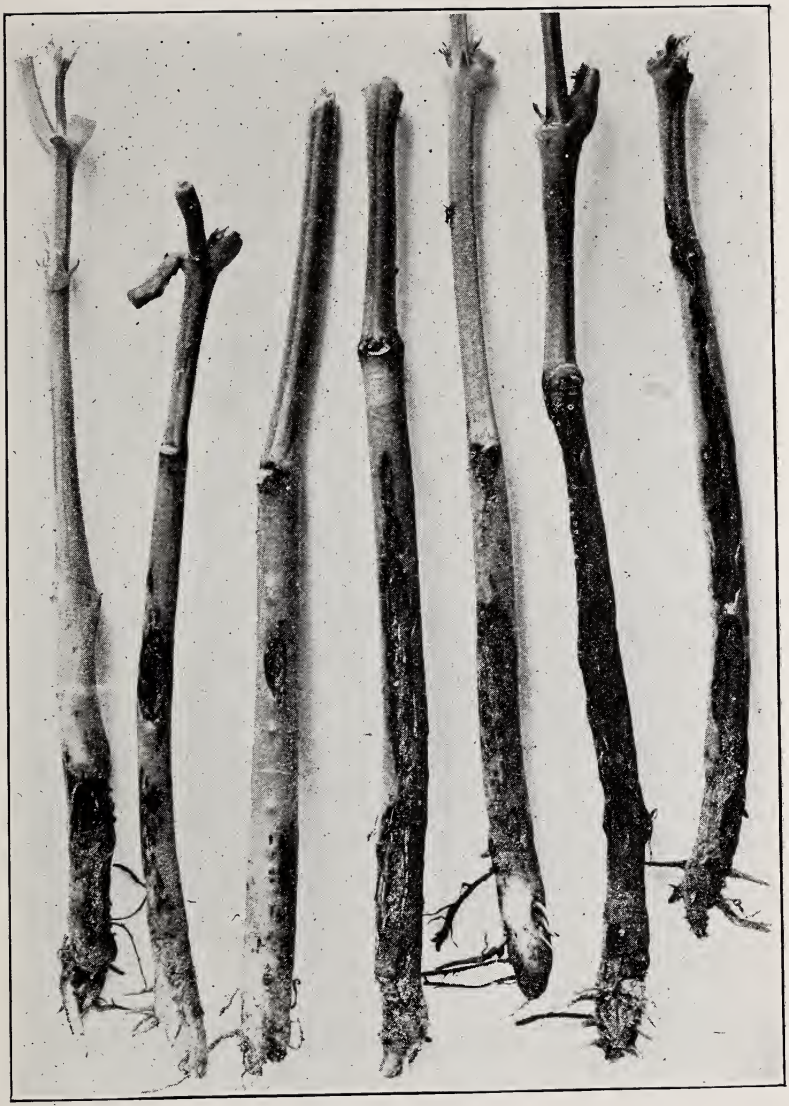

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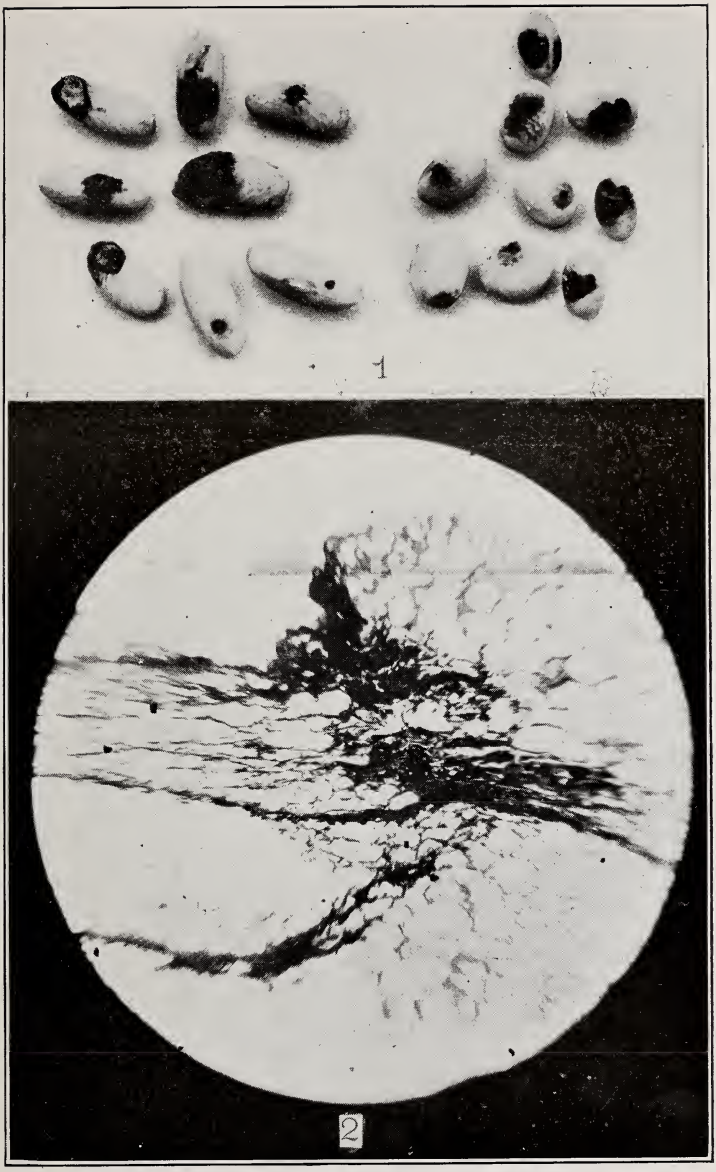


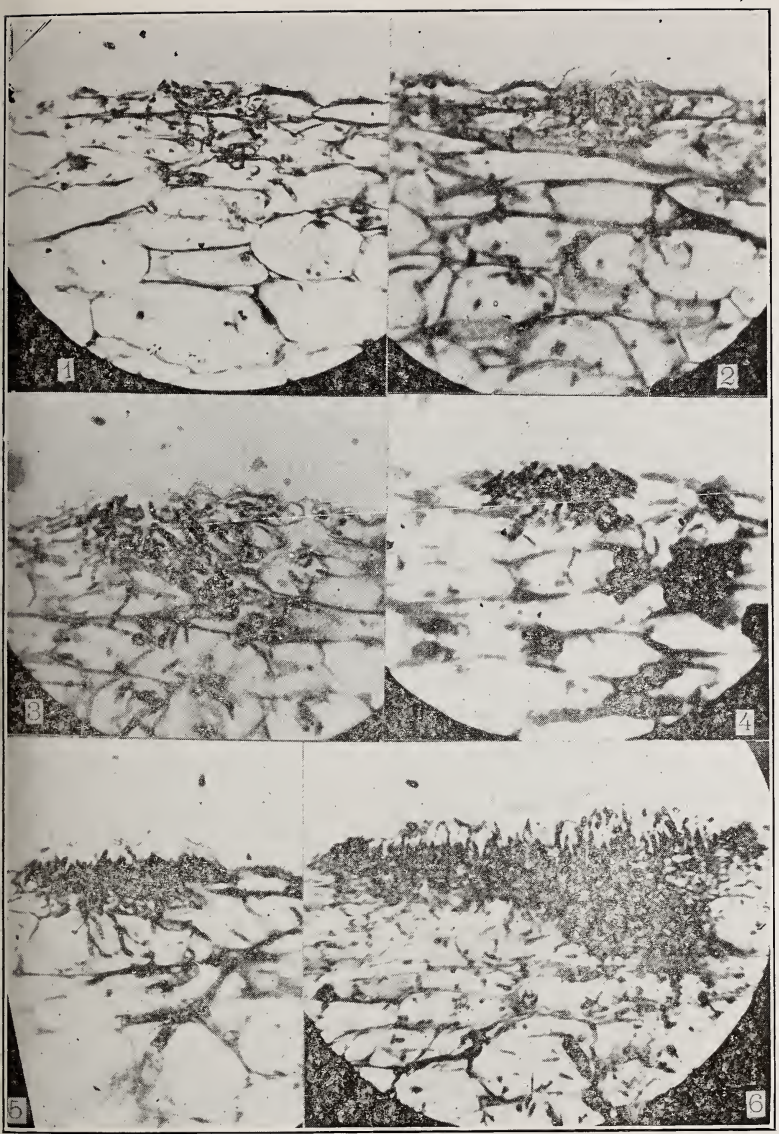
Plate VII.

PLATE VIII.

PLATE IX.

PLATE X.

PLATE XI.



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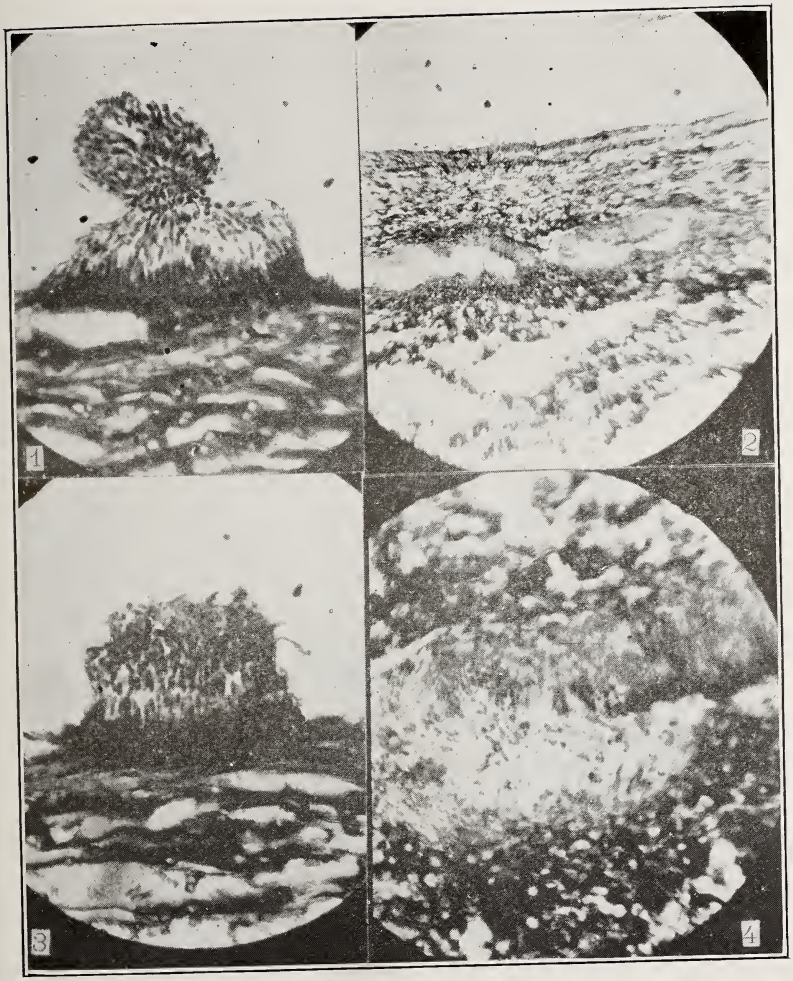

PLATE XIII.

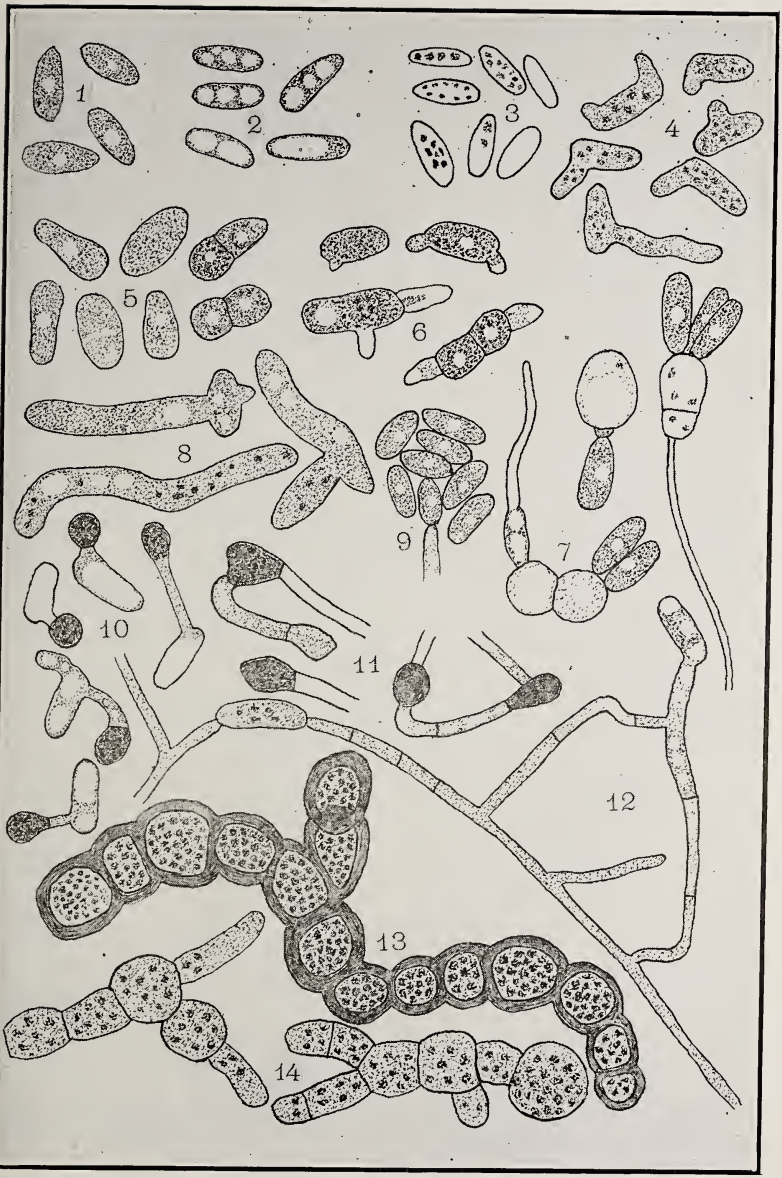

PLATE XIV.